84

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XXXVIII APRIL, 1920 NO. 4

CONTENTS

		PAGE
BERRY, S. STILLMAN	*Light Production in Cephalopods, II*	171
GOODRICH, H. B.	*Rapidity of Activation in the Fertilization of Nereis*	196
DANCHAKOFF, VERA	*Immunity and the Power of Digestion*	202
HARMAN, MARY T.	*Chromosome Studies in Tettigidae. II. Chromosomes of Paratettix BB and CC and their Hybrid BC*	213
BRIDGES, CALVIN B.	*White-Ocelli—An Example of a "Slight" Mutant Character with Normal Viability*	231
ISHII, O.	*Observations on the Sexual Cycle of the Guniea Pig*	237

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand, London, W. C.

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor. the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XXXVIII. *April, 1920.* *No. 4.*

BIOLOGICAL BULLETIN

LIGHT PRODUCTION IN CEPHALOPODS, II.

AN INTRODUCTORY SURVEY.

S. STILLMAN BERRY,

REDLANDS, CALIFORNIA.

CONTENTS.

PAGE

5. Color and Intensity of Light 172
6. Distribution of Photophores on Animal 174
   On Eyeball 175
   In General Integument of Body 176
      On Mantle 176
      On Head 176
      On Arms 176
      On Funnel 177
      Around Eye-openings 177
      On Fins 177
   In Arms 179
   In Tentacles 180
   Intrapallial 180
      Anal 181
      Branchial 181
      Gastric 181
      Axial 181
   At Tip of Body 182
7. Structure of Photogenic Organs 183
   Diversity 184
   Component Structures 185
   Duplex Organs 185
8. Polymorphic Nature of Photogenic Organs 186
   Genera with Isomorphic Photophores 187
   Genera with Dimorphic Photophores 187
   Genera with Trimorphic Photophores 187
   Genera with Polymorphic Photophores 187
9. Systematic Significance of Photogenic Organs 189
10. Probable Polyphyletic Origin of Photogenic Organs 190
11. Concluding Note 191
12. Summary 192
13. Literature 193

5. Color and Intensity of Light.

But little is known concerning the color, particularly what may permissibly be termed the *intrinsic* color, of the light produced by cephalopods, in fact next to nothing of any of its fundamental physical qualities. This of course follows as a natural corollary of the scanty nature of the recorded human observations of these animals in the living state. Such as they are the appropriate data gleaned from the preceding section of this paper are briefly tabulated.

Vérany's observations previously quoted are a little ambiguous and it is not just evident whether the "sapphire blue" and "topaz yellow" rays which he describes with such naïve enthusiasm for the photophores of *Histioteuthis* apply to the result of their functional activity at night, or merely to their ordinary brilliant coloration in the daytime. The fact that he was "blinded" would seem to indicate the former.

Table IV.

Color of Light in Cephalopods.

Species.	Date of Published Observation.	Color of Light.
Œgopsida:		
Family Lycoteuthidæ,		
Lycoteuthis diadema (Chun)	Chun, 1902.....	ultramine, sky blue, ruby red, pearly white.
Family Enoploteuthidæ,		
Watasenia scintillans (Berry) ...	Watasé, 1905, 1912.	
	Ishikawa, 1913 ..	clear blue.
	Sasaki, 1914.....	Prussian blue to purplish.
Family Histioteuthidæ,		
Histioteuthis bonellii (Férussac)..	Vérany, 1851.....	?
Family Ommastrephidæ,		
? "*Loligo sagittatus* Lamarck" ..	Giglioli.....	?
Family Cranchiidæ,		
Cranchia sp.....	Holder, 1887.....	?
Myopsida:		
Family Sepiolidæ,		
Sepiola sp. (*rondeletii* Leach ?)..	Marchand, in Meyer, 1906.....	?
Inioteuthis japonica Verrill.....	Sasaki, 1914.....	"cobaltish."
Heteroteuthis dispar (Guppell)...	Meyer, 1906.....	pale greenish.
	Dahlgren, 1916....	blue green.
Octopoda:		
? *Polypus* sp.....	Darwin, 185.....	?
? small octopod.....	Giglioli.....	"pale whitish."
Tremictopus gracilis (Souleyet).	Souleyet.....	?

Of the subsequent observations, only a few trouble to specify the apparent color of the light rays. I use the word apparent advisedly, not alone because of the ever-present subjective considerations by which one and the same ray may yield diverse impressions to different persons at the same time and under the same conditions, or to the same person at different times or under different conditions, but also because there is evidence that the original color values of the light rays may suffer modification, either by reason of the physical features of some of the supervening tissues of the photophore itself, or by the interposition of the chromatophoric color screens to which attention has already been drawn.

The extent to which the brilliantly varied illumination which was described by Chun for *Lycoteuthis diadema* is due to such considerations as these, rather than to differences inherent in the light rays produced by the respective organs is therefore a matter for considerable speculation. In this species Chun (:03, pp. 569–570; :03*a*, p. 81; :10, p. 50) described the light of the central organ in each subocular series as "marvelous ultramarine blue," of the anterior axial organ as "sky blue," of the two anal organs as "ruby red," of the remaining organs as "snow-white" or "pearly." But it should be remembered that no matter what other rays may have suffered absorption to result in the described effect on the human eye, no sort of screen or filter could manufacture those which evidenced themselves and they must therefore have been produced within the photogenic tissues. If, as in all other luminous organisms which have been subjected to examination, this is still a relatively efficient and therefore "cold" light, the question is yet before us whether the "ruby red" rays of *Lycoteuthis* are none the less as "cold" as the ultramarine and blue ones, or as the blue-green luminescence of the firefly. The biochemist and biophysicist have here a tempting field, once the technical biological difficulties of securing and handling the animals can be fairly overcome.

The light of the luminous secretion of *Heteroteuthis dispar* is described by Meyer (:06, p. 389) as "pale greenish," and by Dahlgren (:16, p. 71) as "the usual blue-green of luciferine when burning outside the body."

Sasaki and other observers of *Watasenia scintillans* describe the light of the large organs at the tips of the ventral arms as purplish or Prussian blue, the body organs appearing "whiter and less luminous" than these. In spite of their absolutely different histological structure, the rays emanating from the integumentary and subocular organs do not here appear to be respectively distinguishable and one wonders whether, in this regard, the observations recorded convey the whole truth.

That such elaborate variety in the size, morphological detail, and possession of accessory contrivances as will shortly be described, must find at least partial expression in differences in the physical qualities (intensity and color) of the resulting light rays, seems as inescapable to the present writer as it did to Chun (:03*a*, p. 81). And on the whole the scanty evidence just outlined is in accord, showing that the hues of the light are different, often most strikingly so, not alone as between independent species, but between the organs occupying different situations on the body in one and the same species.

6. Distribution of Photophores on Animal.

The photogenic function in cephalopods is, as has been seen, not a general attribute of the body surface, but is always, so far as is known, localized in the specialized tissue of definitely circumscribed organs disposed in equally definite regions of the body. It therefore becomes appropriate to examine what position or positions on the body these structures have come to occupy. Proceeding accordingly, one is at once struck with the fact that although strong evidence of partiality for certain special situations exists, yet no hard and fast rule may be laid down. The region where the organs occur most commonly seems to be by all means the surface of the ventral hemisphere of the eyeball. Photophores are found in this position in most (probably all) of the Cranchiidæ, in *Enoploteuthis*, *Abralia*, *Abraliopsis*, *Watasenia*, *Asthenoteuthion*, *Pyroteuthis* and *Pterygioteuthis* of the Enoploteuthidæ, in all the Lycoteuthidæ, in *Lampadioteuthis*, in *Ctenopteryx*, and in *Chiroteuthis*,—at least some 25 and more probably around 29 of the entire 44 photogenic genera in the suborder Decapoda. Most of the cranchiid genera, comprising, so far

as known, the entire subfamily Taoniinæ, are peculiar in that the subocular photophores are reduced to one or at most two organs which are frequently so large as to cover nearly the entire lower surface of the eyeball. When two such are present they are semicircular or more or less crescentic in outline, the smaller or anterior organ fitting into the concavity of the larger. The eye-organ in *Ctenopteryx* is a single large falciform structure. In most genera, however, the subocular photophores are smaller and more diffuse in their arrangement, the commonest system being an alignment in a simple, bead-like, longitudinal series on the ventral periphery of the eyeball. Curiously enough, the series usually includes organs belonging to two or more diverse structural types. Such is the arrangement to be found in *Lycoteuthis*, *Nematolampas Abralia*, *Abraliopsis*, *Watasenia* and *Enoploteuthis*, the last-named genus having nine or ten organs on each eye, all the other genera five. *Liocranchia* and *Pyrgopsis* have four organs similarly located, but all of one type. *Chiroteuthis picteti* and *C. imperator* are figured by Chun as having three longitudinal chains of isomorphic organs, 22 to 29 in all, upon each eye. In the latter species he found the number to be somewhat variable, which is an unusual circumstance with the subocular organs. This is a particularly striking fact when the remaining five genera having this type of photophore are considered. In all of these, namely, *Lampadioteuthis*, *Pyroteuthis*, *Pterygioteuthis*, *Cranchia* and *Leachia* the photophores of the eyes, varying in number from four in *Lampadioteuthis* to fifteen in *Pterygioteuthis giardi*, have lost their simple serial arrangement, and the individual organs are scattered to a greater or less degree over the lateral as well as the ventral region of the eyeball. Their distribution thus becomes highly irregular, yet it is almost always absolutely definite and practically invariable within the bounds of each single species. *Chiroteuthis veranyi*, as described by Chun, is unique in having two large bands of photogenic tissue on the ventral convexity of each eye, accompanied by a few small isolated photophores of the more ordinary form, by the coalescence of a number of which they perhaps originated. Since the genera possessing subocular organs are all œgopsid, it follows that

these photophores are covered by the double fold of the integument which forms the eyelid, and consequently in preserved specimens are often invisible without partial dissection. But in the Cranchiidæ the overlying membranes are thin, transparent, and very insufficiently equipped with chromatophores, so that in good specimens the organs may be clearly seen from the exterior. And likewise in certain other groups such as *Enoploteuthis* and the Abralioid genera, we find a delicate, transparent, elongate-oval "window" in the integument, nearly or entirely free of pigmented chromatophores, and overlying that portion of the eyeball where are borne the photogenic organs. There can be little doubt that this functions in aid of the latter by facilitating the passage of their rays.

The next most frequent topographic type of photophore to be met with comprises those occurring in the general integument of the body, primarily on the mantle, head, and arms. A remarkable peculiarity of the integumentary organs is that they, like the subocular photophores, are generally confined to the ventral aspect and this circumstance has given rise to some interesting theories regarding the origin and ecologic significance of the whole phenomenon of light production in this group of animals. Some writers have gone so far as to state that the distribution of these organs is *entirely* ventral, but this is not in strict accord with the facts, there being a few scattered photophores on the dorsal aspect of the mantle in such forms as *Abralia astrolineata* and most of the Histioteuthidæ, while Verrill's figures show them to be quite as strongly developed in this region in his *Mastigoteuthis agassizii*[14] as they are below. Certain other species of *Mastigoteuthis* have them in plenty on the dorsal surfaces of the fins, even if not upon the body proper. Again in Professor Joubin's anomalous *Melanoteuthis* the supposed photophores are entirely dorsal. The possibly photogenic tubercles of *Mastigoteuthis cordiformis* should likewise be recalled in this connection, and finally the presence of photophores on the dorsal arms of *Nematolampas* and *Benthoteuthis*. But even as many exceptions as this serve principally to accentuate the prevalence of the rule. In some genera the integumentary organs are developed on the

[14] *Bull. Mus. Comp. Zoöl.*, V. 8, Pl. I., 1881.

ventral surface of the mantle only (*Ancistrocheirus*, *Hyaloteuthis*, and, according to Chun, though he is controverted by other writers, *Chaunoteuthis*). In others (*Eucleoteuthis*) they occur on the ventral surface of the head as well. In *Calliteuthis*, *Histioteuthis* and some species of *Mastigoteuthis* they are found not only on the mantle and head, but on the aboral surfaces of the ventral and ventro-lateral arms. In *Mastigoteuthis agassizii* they are figured as occurring even on the tentacle stalks, as they do likewise in *Thelidioteuthis*, although this would appear to be an unusual situation for organs of the integumentary type. In the former of these two genera they are numerous in the integument of the head, arms, and mantle as well as the tentacles; in the latter, they are less numerous and although found along the outer side of the tentacles, occur elsewhere only on the ventral aspect of the mantle and head, where they have a very regular and characteristic arrangement. Finally in a number of well-known genera (*Enoploteuthis*, *Abralia*, *Abraliopsis*, *Watasenia*, *Mastigoteuthis*), integumentary organs are plentifully distributed in indefinite number over the entire ventral aspect of mantle, head, arms, and funnel.

On the fins these organs appear less frequently, but they are described as occurring dorsally in several species of *Mastigoteuthis*, and in one (*M. talismani*) on their ventral faces.

In a number of species there is a particular development of the integumentary photophores in the neighborhood of the eyes, usually in the form of a circlet around the margin of the lid opening, and such a circlet may occur, as in *Enoploteuthis*, *Abralia*, *Abraliopsis* and *Watasenia*, in addition to a well developed series of subocular organs. As a general rule, and certainly in the four genera named, the organs comprising this circlet are not to be distinguished from those of the general integumentary surface save by their peculiar arrangement and position. In the Histioteuthidæ, however, comprising the genera *Histioteuthis* and *Calliteuthis*, a most singular modification of this feature is encountered. A peculiar attribute of these genera is that, probably without exception, all the species have the left eye enormously more developed than the right, so much so in fact that a strong lateral torsion or displacement of the entire,

in both genera relatively enormous, head is produced, which would seem to render it a physical impossibility for the animal to propel itself in a straight path without recourse to spiral movement or some violent sort of counter twisting. This asymmetry extends quite inexplicably to the photogenic organs, inasmuch as the "normal" right eye has a well developed circlet of photophores surrounding the lid opening as above described, while the Brobding nagian left eye has the photophores of its circlet not only pulled farther apart by the distention of the lid, but its every component reduced almost to a rudiment, some of them quite atrophied, or they may even be, as Sasaki has stated for *Calliteuthis separata*,[15] absent entirely. It seems as though from the very nature of the case there must be some correlation between such pronounced asymmetry and the habits of the animals, but no reasonable explanation of what might be necessary to bring about or to render advantageous such an anomalous condition seems ever to have been suggested. In *Mastigoteuthis glaukopis* there is no circumocular circlet of photophores, but a single photogenic organ is described as occurring in the ventral edge of each lid sinus.

In a few species the integumentaty photophores are few and consequently definite in number and position (*Ancistrocheirus*, *Thelidioteuthis*, *Hyaloteuthis*, *Eucleoteuthis*). This is probably true also of the very young or larval stages of all the species possessing photogenic organs, but in adults of most species, though still continuing to retain more or less evidence of the primal bilateral symmetry, they are apt to increase to such an extent as to become practically or quite impossible of separate identification and enumeration and thereupon show little constancy in either number or position.

Eucleoteuthis is a genus which deserves discussion by itself. It is unique among known cephalopods in that its photogenic organs instead of forming small rounded or ovoid capsules as in practically all the other genera, are developed as a pair of narrow, more or less interrupted stripes or bands of photogenic tissue extending along the ventral aspect of the mantle for nearly its entire length. A small oval tract of similar tissue flanks the

[15] *Journal College Agriculture Tohoku Imperial University*, V. 6, p. 137, 1915.

outer side of each band at its anterior end, and, in the type species at least, there is a pair of somewhat larger, transversely ovoid photogenic areas on the head at the base of each ventral arms. That these curious tracts should be classified with the remaining organs here collectively referred to as integumentary is by no means certain.

The arms are a favored situation for photogenic organs. The extension along their outer surfaces of the ordinary integumentary photophores in the case of such genera as *Enoploteuthis*, *Abralia*, *Abraliopsis*, *Watasenia*, *Calliteuthis*, *Histioteuthis*, and certain forms of *Mastigoteuthis*, has already been noted. In addition to this certain special types of organs are sometimes developed. One of the generic characters of *Chiroteuthis* is the presence of a series of conspicuous dark photophores along the oral aspect of each of the greatly enlarged ventral arms. *Nematolampas* has a small dark photophore embedded in the extreme tip of each arm of the two dorsal pairs. Not only this but each arm of the third pair bears immersed in its tissues along the outer margin a series of plainly visible photogenic organs which continue as the principal component of a long, chain-like, filamentous extension of the arm which in life must extend like a string of fiery beads far in advance of the animal. There are in excess of thirty individual organs in each chain, but the true number may be much greater as no specimens of the species still retaining these extraordinary structures entirely in their pristine state have yet been captured. In *Abraliopsis* and *Watasenia*, genera so closely allied to one another that one could with about equal ease be regarded as but a subgenus of the other, there are three large, black, bead-like photophores, with perhaps some smaller, more rudimentary ones, in close juxtaposition at the tips of each ventral arm. As previously related, these are known to give forth a brilliant light. Rudiments of similar organs correspondingly situated are known in at least one species of *Abralia*, another nearly related genus. This is *A. astrolineata* Berry of the Kermadec Islands. The curious deep-sea *Benthoteuthis* has a single photophore on the outer periphery of each arm of the three dorsal pairs near the base, and none elsewhere on the body, an arrangement wholly unlike that met with in any other cephalopod.

Until very recently photophores on the tentacles have been supposed to be of rare occurrence, but it has lately been shown that they do actually so exist in quite a number of diverse forms, having tended to escape observation by reason of being embedded so deeply in the fundamental substances of the tentacle stalk as to be quite invisible in preserved material unless thoroughly cleared or otherwise specially treated. In *Pyroteuthis* Vivanti and Mortara have recently established the presence of a series of four such organs in the stalk of each tentacle. I had not only independently made the same discovery in material from both the Atlantic and Pacific, but had likewise found that there is yet a fifth tentacular organ present, and that the same condition obtains as well in the nearly related genus *Pterygioteuthis*. *Lycoteuthis* and *Nematolampas* have two such organs in each tentacle stalk. *Lampadioteuthis* is unique in possessing not only a series of four photophores embedded in the stalk proper, but in addition tucked away at its very base, a single large spherical organ of peculiar structure which is quite invisible without extraction of the entire tentacle from its socket. Conspicuous tentacular photophores are also shown in Verrill's figures of his *Mastigoteuthis agassizii*,[16] but the inference seems to be, as has been indicated above, that these are simply of the ordinary integumentary type, as seems to be true also of the tentacular photophores in the genus *Thelidioteuthis*.

We now come to the class of photogenic organs which is perhaps the most distinctive of the Cephalopoda as compared with other luminous animals, and which, next to the subocular photophores, exhibits the most general distribution within the group. Included here are a large array of very diversely constructed photophores found in quite various situations upon the visceral mass within the pallial chamber. These one and all, however, except in the case of those myopsids which eject their luminous secretion through the funnel, must naturally depend in life upon the more or less complete transparency of the mantle tissues to permit the unobstructed emanation of their beams. In preserved specimens, as would be expected, they can rarely be seen without laying open the pallial chamber, whereupon they are

[16] *Bull. Mus. Comp. Zoöl.*, V. 8, 1881, pl.1.

generally easy to distinguish, many of them being of unusual size and often of conspicuous coloration, while the situations which they occupy are peculiarly limited and, within a given species, constant. By reason of this last fact the intrapallial organs may readily be subclassified into four series, (1) anal, (2) branchial, (3) gastric, and (4) axial. Such a classification, too, in spite of its obviously superficial foundation, is a convenient one. That it is at the same time in all respects a natural or phylogenetic arrangement is probably not true, and it will no doubt be greatly improved upon by the first worker who takes up the relationships of these organs in any sort of adequate detail.

The term anal organs is misleading, but has become so well established in the literature that I use it pending the invention of a more appropriate term. The photophores so classified appear usually as a pair of quite large, often very brightly colored organs of rounded or ovoid outline, lying on the ink sac on either side of the rectum, with which they would otherwise appear to have no particular connection. Being often situated just back of the funnel, or sometimes almost within it, they are therefore sometimes termed the siphonal photophores, a name which in its turn is open to objection as inappropriate to the actual morphological relationships involved. Anal organs occur in a considerable number of little related genera, and the discharging photophores of the luminous Sepiolidæ are noteworthy for occupying an analogous situation.

The branchial organs are always paired, being situated one near the base of each gill. They are confined, so far as known, to the Lycoteuthidæ, Lampadioteuthidæ, and the pterygiomorph section of the Enoploteuthidæ.

The gastric and axial organs are classed together by most writers under the general term abdominal, but I prefer to separate the mesially situated, unpaired organs, which are often extended into a considerable series in the hinder portion of the mantle cavity, from the paired organs which sometimes occur near the middle of the body on either side of and often in close association with the anteriormost of the axial organs. There is evidence that in at least some genera the division here postulated into the

paired gastric and unpaired axial organs is founded upon a good morphological as well as merely topographical basis, but at the same time it is impossible to emphasize too strongly that we are here dealing primarily with the mere somatic distribution of the organs, and not with a true genetic classification based on the embryology or finer anatomy, save where the latter becomes incidentally involved. The need for this qualification has no doubt already been patent to the reader from the foregoing discussion.

Of the dozen genera listed in the synopsis as possessing intrapallial photophores, only *Heteroteuthis*, *Sepiola*, *Euprymna*, *Chiroteuthis* and *Corynomma*[17] are described as having anal organs only, a single pair or organ formed by the fusion of a pair being present in each instance. *Lampadioteuthis* has paired anal and branchial organs (the latter very large) and a single posterior axial organ. *Pterygioteuthis* has paired anal and branchial organs, and four axial organs, the most anterior of which is vastly the largest, the most posterior very minute and pushed far down past the fins into the sharp-pointed tip of the body. *Pyroteuthis* has a quite similar illumination system, but the foremost axial organ is more anterior in position, is only a little larger than the others, and is flanked on each side by a small gastric organ. *Lycoteuthis* and *Nematolampas* have a single pair each of anal, branchial and gastric organs as above, a small anterior axial and a very large posterior axial organ. Branchial, gastric and anterior axial organs are placed at about the same transverse plane so that they form a belt of fiery jewels near the middle of the body. *Onychoteuthis* (*banksii*) is unique in having but two large unpaired photophores, both of which are intrapallial and lie upon the ink sac in the median line, one very large and ensconced in a specially constructed depression on the ink sac proper, the smaller upon the narrow, neck like, anterior portion of the sac.

The minute unpaired organs which have been mentioned as occurring in the spine-like tip of the body in *Pterygioteuthis* and *Pyrotheuthis* are probably correctly interpreted as but the terminal members of an unusually developed axial series. Lo-

[17] Chun rather doubtfully adds *Octopodoteuthis* to this list.

cated in the same general region as these, and, by their appearance, seeming to bear a closer relation to the intrapallial organs than to the other systems outlined, yet scarcely to be regarded as lying actually within the mantle cavity, are the conspicuous paired photophores placed at the extreme posterior tip of the body in *Nematolampas*. *Lycoteuthis* does not possess them. They stand in a class quite by themselves at present, but if the peculiar swellings to be noted in the same situation in certain species of *Abralia* are susceptible of a photogenic interpretation, or if Chun's identification of the posterior disk of *Spirula* as a luminous organ be accepted, a further extension of this division of the classification is afforded.

7. Structure of Photogenic Organs.

Another most remarkable feature of the development of photogenic systems in Cephalopoda is, so far as I am aware, the quite unparalleled variety of structural type manifested by their constituent organs. It is entirely beyond the scope of this paper to enter into any extended account of the histological detail, but it will be useful to call attention to at least a few of the main features. Suffice to say that since the first observations on the finer morphology of cephalopod photogenic organs made by Joubin in 1893, a most bewildering variety of structure within the confines of this single, narrowly limited group of animals has been brought to light, ranging all the way from the simple discharging glands of the luminous myopsids, and the lump of photogenic tissue which forms the proximal photophore in the tentacle of *Lycoteuthis*, through almost innumerable intermediate types, to the astonishingly complex bull's-eye lanterns of *Abraliopsis* and the mirrored searchlights of the Histioteuthidæ. Each species has in fact its own peculiar modifications and sometimes many of them. The histology of all affords a fruitful field of investigation, which, with all due respect to the fine work of Chun, Hoyle and Joubin, we can truly say has been hardly skimmed. This is especially true of the embryology and he who attempts to work out the origin and homologies of even the simplest of these organs will have a virgin field.

Cephalopod photophores appear only rarely to be made up

of masses of photogenic tissue without accessory structures (intrapallial organs of Sepiolidæ; proximal tentacle organs of *Lycoteuthis* and *Nematolampas*; eyelid organs of *Mastigoteuthis glaukopis*). As a general rule they are more or less complicated.

The principal division of the organs on morphological grounds is that already noticed which places the discharging glands of the Sepiolidæ on the one hand,—the enclosed glands of the remaining photogenic genera on the other. The latter it is again possible to roughly separate into three types: the no doubt relatively primitive invaginated epithelial organs of which the subocular photophores of *Cranchia*, *Liocranchia* and *Leachia* are interesting examples, band-like expanses of photogenic tissue as in *Eucleoteuthis*, and the spherical, ovoid or discoid organs, often provided with the most extensive array of accessory mechanisms, which are found in most of the other genera.

The organs of the last mentioned class in their highest development attain to an almost unbelievable degree of complexity To the primary photogenic tissue, with its invariably abundant blood and nerve supply, are here added more or less efficiently developed reflector mechanisms, pigment cups, lenses, diaphragms directive muscles, mirrors, windows, color screens,—even in some cases accessory photophores, giving rise to the puzzling "double organs" which are met with now and then in the most dissimilar situations, so that their purpose and manner of functioning is left even more than it otherwise would be a complete enigma. In some cases only certain ones of these accessory structures are developed, in other cases nearly all, as in the miniature searchlights which yield such beautiful microscopic preparations in the integument of the Abralioid and Histioteuthid forms. Space will not permit a complete description, but the presentation of these various accessories in outline form will give an idea their wonderful variety and serve likewise as a convenient summary. The student desiring more detailed information is referred to the works cited in the bibliography, particularly those of Joubin ('93, '93*a*, '93*b*, '93*c*, '94, '95, :05, :05*a*), Hoyle (:02, :04, :09), Meyer (:06, :08), Vivanti (:14), and the beautiful memoirs of Chun (:03*a*, :10).

TABLE V.

COMPONENT PARTS OF THE CEPHALOPOD PHOTOPHORE.

I. Primary (photogenic tissue).
   1. Photogenic cells.
   2. Veins and arteries.
   3. Nerves.
   4. Connective tissue.

II. Secondary (accessory structures).
   1. Pigment cup (almost always present, but sometimes lacking where photophore is surrounded by other pigmented tissue, as the ink sac or eyeball).
      (*a*) Chromatophores.
      (*b*) Specially modified pigment cells (an adaptation of preceding ?).
   2. Reflector, or Tapetum.
      (*a*) Nucleated cells.
      (*b*) Fibers.
   3. Scale cells, or "Schuppenzellen" of Chun.
      (*a*) as reflector.
      (*b*) as lens or cornea.
      (*c*) in photogenic tissue.
   4. Lens.
      (*a*) Fibrillar.
      (*b*) Cellular.
         (1) Connective tissue.
         (2) Modified mantle musculature.
   5. Diaphragm.
      (*a*) Chromatophores.
      (*b*) Muscles.
   6. Window.
   7. Mirror.
   8. Accessory photophores ("double organs").

The duplex photophores deserve a further word. These comprise two separate masses of photogenic tissue so closely associated together that the conclusion seems unavoidable that in some way they function in common. Organs of this type seem to have been first discovered by Chun, who described them in some detail for a number of species. There is small doubt that histological examination will show the occurrence of similar organs in many other instances also. The double crescentic subocular photophores of certain Cranchiidæ have been briefly described on an earlier page. *Lycoteuthis* (and most probably *Nematolampas* also)[17] possesses a number of duplex organs, the

[17] *Nematolampas* certainly agrees in having the terminal subocular photophores equipped with an accessory photophore. The other organs mentioned have not yet been investigated.

distal organs of the tentacles, the terminal members of the subocular series, and the gastric organs, all being of this category. In the gastric organs, the respective masses of photogenic substance, though entirely distinct from one another, are contained within the same capsule. In the case both of these and the terminal subocular organs, which are separated, the accessory photophore lies beneath the principal one and the rays which emanate from it must accordingly pass through the latter if they are to have egress at all. In *Pterygioteuthis* the branchial organs are duplex, the accessory organ being contained a little to one side of its principal, but still within the same pigment cup.

8. Polymorphic Nature of Photogenic Organs.

The question is now very near, whether so many simple and elaborate morphological types of light-producing organs have any especially closer genetic relationship to one another where they are found within one and the same species or genus. And this leads easily to another, whether the photophores of any given species exhibit such manifold structural diversity as to render improbable their ultimate reduction to a single primordial type. The affirmation of this latter question implies the negation of the former, and I think we may certainly say that this seems most truly to express the facts as we have them. The accompanying table (Table VI.), which it has seemed worth while to elaborate upon the basis of the interesting outline given given by Chun, shows that whereas about a third of the genera cited each possess photophores belonging to a single general type, nearly as many have strongly dimorphic photophores, and an even greater number have trimorphic or polymorphic organs. It is nothing unusual therefore to find organs of extreme simplicity functioning as components of the same photogenic system which contains also organs exhibiting the most varying degrees of complexity in structural plan. While this seems to take place almost in hit or miss fashion, I think it may be taken as a general statement of fact that those species having a relatively abundant development of integumentary photophores distributed over the body generally fail to evolve a great variety of other types, the Abralioid genera providing the nearest to an

exception to this rule (see Table VII.). Those species showing the richest development of structural type in general are the ones which depend upon intrapallial rather than integumentary organs to serve the light producing function. Here there is sufficient divergence among the various organs as to discourage almost at a glance any attempt to homologize them on the basis of reference to a single primal type. Not only their diversity, but their extremely sporadic appearance in connection with organs and tissues of heterogeneous origin, is strongly inhibitive of any such view.

TABLE VI.

POLYMORPHISM IN CEPHALOPOD PHOTOPHORES.

I.	Genera with Isomorphic Photophores.	
	Thelidioteuthis	Integumentary.
	Histioteuthis	"
	Calliteuthis	"
	Bentholeuthis	On arms.
	Mastigoteuthis	Integumentary.
	Cranchia	Subocular.[18]
	Liocranchia	Subocular.
	Pyrgopsis	"
	Henselioteuthis	"
	Bathothauma	"
II.	Genera with Dimorphic Photophores.	
	Enoploteuthis	Integumentary; subocular.
	Leachia	Subocular.
	Megalocranchia	"
	Crystalloteuthis	"
	Toxeuma	"
	Taonidium	"
	Corynomma	Subocular (?); intrapallial.
III.	Genera with Trimorphic Photophores.	
	Abralia (except *A. astrolineata*)	Integumentary; subocular (latter dimorphic).
	Chiroteuthis	On ventral arms; subocular; intrapallial.
IV.	Genera with Polymorphic Photophores.	
	Lycoteuthis	In tentacles; subocular; intrapallial; 10 types (13 if 3 types of accessory organs are counted separately).
	Nematolampas	In arms; in tentacles; subocular; intrapallial; at tip of body; probably 12 or 13 types (15 or 16 if accessory organs are counted separately).
	Lampadioteuthis	In tentacles; subocular; intrapallial;—probably 7 or 8 types.
	Abralia astrolineata, *Abraliopsis*, *Watasenia*,	Integumentary; tips of ventral arms; subocular (dimorphic).
	Pterygioteuthis	Tentacular; subocular; intrapallial;—8 types.
	Pyroteuthis	Tentacular; subocular; intrapallial;—probably 8 or more types.

[18] Unequal in size, but showing clear structural evidence of homology.

TABLE VII.

DETAILED OUTLINE OF OCCURRENCE OF POLYMORPHIC PHOTOPHORES IN CEPHALOPOD GENERA.

	Lycoteuthis diadema.	*Nematolampas regalis*	*Lampadioteuthis megaleia.*	*Abralia astrolineata.*	*Abraliopsis* sp.	*Watasenia scintillans*	*Pyroteuthis margaritifera.*	*Pterygioteuthis gemmata.*	*Pterygioteuthis giardi.*
Integumentary:									
Dorsal surface of mantle	–	–	–	+(1)	+(1)	+(1)	–	–	–
Ventral surface of mantle	–	–	–	+	+	+	–	–	–
Ventral surface of funnel	–	–	–	+	+	+	–	–	–
Ventral surface of head	–	–	–	+	+	+	–	–	–
Ventral arms	–	–	–	+	+	+	–	–	–
Circumocular	–	–	–	+	+	+	–	–	–
Subocular:									
Ventral periphery	10(3)	10(3)	6(2?)	10(2)	10(2)	10(2)	24(3)	28(4)	30(4)
Lateral	–	–	2	–	–	–			
Arms:									
Tips of dorsals	–	2	–	–	–	–	–	–	–
Tips of dorso-laterals	–	2 (1?)	–	–	–	–	–	–	–
Ventro-laterals	–	62+(1)	–	–	–	–	–	–	–
Tips of ventrals	–	–	–	6(1)	6+(1)	6+(1)	–	–	–
Tentacles:									
At base	–	–	2(1)	–	–	–	–	–	–
Along stalk	4(2)	4(2)	8(1)	–	–	–	10(1)	?	10(1)
Intrapallial:									
Anal	2(1)	2(1)	2(1)	–	–	–	2(1)	2(1)	2(1)
Branchial	2(1)	2(1)	2(1)	–	–	–	2(1)	2(1)	2(1)
Gastric	2(1)	2(1)	–	–	–	–	2(1)	–	–
Axial	2(2)	2(2)	1(1)	–	–	–	4(1)	4(1)	4(1)
Posterior tip of body	–	2(1)	–	?	–	–	–	–	–
Total number photophores	22	90+	23	16+∞	16+∞	16+∞	44	36(+?)	48
Probable number structural types	(10)	(12 or 13)	(7?)	(4)	(4)	(4)	(8)	(8?)	(8)

The photogenic systems of all the species of the eight genera having polymorphic organs are outlined in further detail in Table VII. Those species considered having the mere largest number of photophores are the three Abralioids, occupying the three central columns of the table, but those exhibiting the highest degree of polymorphism are *Lampadioteuthis megaleia*, which has not been investigated histologically but must have not less than seven or eight types of photophores in all, *Lycoteuthis diadema*, with ten types, or thirteen, if the accessory organs are counted in, and *Nematolampas regalis*. *Lycoteuthis diadema*, with the immeasurable advantage of having had its marvelous photogenic properties observed in the living state, is usually cited as the example par excellence of a luminous cephalopod. However, it is evident from sheer morphological grounds that even this wonderful creature must yield the palm to another, if nearly related, genus and species,—the truly amazing *Nematolampas regalis* of the Kermadec Islands. Whether this species will ultimately be found to display all the varied brilliance of the red, white, and blue lights of *Lycoteuthis*, the fact remains that in addition to a complete series of exactly homologous organs, it has an entire battery of pyrotechnic engines of its own, so there is every reason to expect a more rather than a less elaborate illumination. The total number of photophores in this species is in excess of ninety, which are elaborated upon no less than twelve or thirteen different structural principles of uncertain homologies with one another. Counting in the three types of accessory photophores which are to be found in the eight "double" organs (proximal tentacular, terminal subocular, and anal), the total number of types is increased to fifteen or sixteen. Which of the alternative figures quoted is the correct one is still to be established by histological work.

9. Systematic Significance of Photogenic Organs.

It follows almost as a corollary from what has been said in the foregoing sections of this paper that the photogenic system evinces a complex of features of the utmost value to the taxonomist. Of late years ever increasing weight has been given to it, and the presence of constant differences, even though minute, in

its components, is now admitted practically without debate as ample ground for taxonomic discrimination. Where such differences are shown to occur, further differences in the remaining organization seem practically predestined for eventual discovery. Good characters for specific discrimination are to be found, not only in the presence or absence of photogenic organs, but also in their distribution on or within the body, in their number, in their size, and in the veriest details of their intrinsic structure. The taxonomist has in fact few more convenient points of attack in the pursuit of his primary objects of classification and relationship than that afforded by the light organs. And this is exactly what we find, if to somewhat less degree, among the fishes and the few other groups where the photogenic organs have attained some considerable complexity. One can construct a fairly workable taxonomic key based on the photogenic organs alone, for such species as possess them.

10. Probable Polyphyletic Origin of Photogenic Organs.

Before concluding this paper a somewhat general answer may be attempted to a question which has no doubt occurred more than once in the mind of the reader, and which indeed has been touched upon very nearly on more than one occasion—Is photogenesis a primitive function among cephalopods? In other words, are our present day species descended from an ancestral photogenic stem, some branches of which have now yielded up the function? Or has photogenesis arisen several times in this class of animals, possibly to meet altogether diverse conditions or associations in the environment, so that its presence therefore becomes of secondary rather than primary significance?

At first glance the widespread distribution of the function in the great and, comparatively, primitive œgopsid group of cephalopods favors an affirmative answer to our first query. But in reply to this it may be said that the varied pelagic environment of these forms would almost per se favor the development of the light-producing function after a manner which would be hardly likely to hold true among the more littoral Myopsida and Octopoda, the former of which are mainly frequenters of much shallower water than the Œgopsida, the latter hardly ever

pelagic at all, and then generally surface forms or confined to the shallower water like so many of the myopsids.

There are many other arguments which may militate against any theory of monophyletism and as strongly support the contrary view as brought out by the last query above. These, having already been largely elaborated elsewhere or to be dealt with in another connection later on, need be merely summarized here. Such considerations are:

1. The uneven distribution of photogenic organs throughout the entire group, and, as a corollary of this, their appearance in distantly related groups more or less sporadically.

2. The variety and sporadic character of the development of photogenic organs in different regions of the body.

3. The large number of strongly diverse structural types.

4. The evidence from ecological considerations, the distribution upon the body, and similar facts that these organs have arisen in response to very diverse environmental requirements.

How then may one bespeak a photogenic *system*? Exactly as one speaks of a muscular system, or a skeletal system, or a receptor system in almost any animal body. The term is used in the sense not necessarily indicating an aggregation of homologous structures, but an assemblage of organs within a single organic body exhibiting more or less similar or cöordinate physiological reactions, if at times neither in fact phylogenetically nor ontogenetically related.

11. Concluding Note.

This paper is mainly a compilation from the scattered work of other authors. No doubt there are omissions, but the aim has been to present simply a concise summary of the knowledge of this subject which has been gained to the present time. It cannot be too strongly emphasized that not only are many more species of luminous cephalopods likely to be discovered in the future, but some of those now known but not yet recognized as possessing photogenic properties are likely to be revealed as having them. Of the known luminous forms some will no doubt prove to possess luminous organs or properties additional to those described. Bearing all this in mind, if this little paper but fur-

nishes some delving student just a little better base of attack on his problem than might otherwise have been afforded him, its purpose will have been fulfilled.

12. Summary.

1. Light production is an unusually widespread phenomenon in the molluscan Class Cephalopoda.

2. Although unknown in the Order Tetrabranchiata, scarcely developed in the octopod section of the Dibranchiata, and occurring little more than sporadically among the Myopsida, over one half of all described Œgopsida are known to possess photogenic properties.

3. The actual production of light by living cephalopods has been observed only rarely, but in species of sufficiently diverse relationship to confirm the evidence drawn from the morphology and histology of organs found in the remaining species.

4. The light of some species exhibits remarkable brilliance.

5. The color of the light emanating from the respective organs within the same species or in different species may exhibit striking differences in both intensity and quality, but it is not known to what extent this is actually due to inherent diversity in the physical properties of the light rays themselves.

6. Photogenic organs may occur in almost any portion of the body in this group of animals, but the outer integument, eyeball and pallial chamber are the situations most favored. They are often internal and able to function only by reason of the transparency of the body tissues in the living state.

7. The organs are predominantly, but by no means exclusively, ventral in distribution.

8. The organs are strongly polymorphic, even in the same species, varying from comparatively simple bodies of photogenic tissue to the highly complex "searchlight" types.

9. Numerous duplex organs, or organs with accessory photophores, are known to occur.

10. Luminous organs in the Myopsida are usually of the type known as discharging. Those of the other groups are entirely of the enclosed or ductless type.

11. The maximum polymorphism in the photophores of any

single species occurs in *Nematolampas regalis* Berry, from the Kermadec Islands, where the 90 or more organs are elaborated upon 12 or 13 more or less diverse structural principles.

12. The occurrence, distribution, arrangement, and morphological detail of photogenic organs in cephalopods are features of considerable taxonomic importance and yield valuable clues as to the relationship and classification of the genera and species even where still unknown anatomically.

13. The best evidence seems to indicate that the photogenic organs in this group of animals are polyphyletic and more or less sporadic in origin, hence that light production in cephalopods is not an essentially primitive or ancestral function to be regarded as now lost in many members of the group.

13. LITERATURE.[16]

Berry, S. S.

'13 Nematolampas, a Remarkable New Cephalopod from the South Pacific. BIOLOGICAL BULLETIN, v. 25, pp. 208–212, 1 fig., August, 1913.

'13a Teuthological Miscellany No. 1. Zoologischer Anzeiger, Bd. 42, pp. 590–592, October, 1913.

Chun, C.

'00 Aus den Tiefen des Weltmeeres. Jena, 1900.

'03 Aus den Tiefen des Weltmeeres. Zweite Auflage, Jena, 1903.

'03a Über Leuchtorgane und Augen von Tiefsee-Cephalopoden. Verhandl. d. deutschen Zoologischen Gesellschaft, 1903, pp. 67–91, text figs. 1–14, 1903.

'10 Die Cephalopoden. 1. Teil: Oegopsida. Wissenschaftliche Ergebnisse d. deutschen Tiefsee-Expedition Valdivia, Bd. 18, pp. 1–402, 2 pls. and 32 figs. in text, Atlas of 61 pls., 1910.

'13 Cephalopoda. Report Scientific Results "Michael Sars" North Deep Sea Expedition, 1910, V. 3, pt. 1, Zoölogy, pp. 1–28, text figs. 1–11, pl. 1–2, 1013.

Dahlgren, U.

'16 The Production of Light by Animals. Journal Franklin Institute, Feb.-April, 1916, pp. 1–75, text figs. 1–20.

Darwin, C.

'60 Journal of Researches into the Natural History and Geology of the Countries Visited during the Voyage round the World of H. M. S. "Beagle" under the Command of Captain Fitz Roy, R.N. 8vo, pp. 1–551, illustrated, London, 1913 (first edition May, 1860).

Holder, C. F.

'87 Living Lights. A Popular Account of Phosphorescent Animals and Vegetables. 8vo, 127 p., 28 pls., New York, 1887.

[16] A more exhaustive bibliography of the subject is in preparation for a later paper of this series.

Hoyle, W. E.

'02 The Luminous Organs of *Pterygioteuthis margaritifera*, a Mediterranean Cephalopod. Memoirs and Proceedings Manchester Literary and Philosophical Society, V. 46, No. 16, pp. 1–14, text fig. 1–6, June, 1902.

'04 Reports on the dredging operations off the west coast of Central Americ- . . . by the . . . "Albatross," etc. Reports on the Cephalopoda. Bula letin Museum Comparative Zoölogy, V. 43, pp. 51–64, pl. 9–10, March, 1904.

'08 Address to the Zolöogical Section, British Association for the Advancement of Science, Leicester, 1907. Report British Association Advancement Science 1907, pp. 520–539 [1–20], text fig. A–D, 1908.

'09 The Luminous Organs of Some Cephalopoda from the Pacific Ocean. Proceedings 7th International Zoölogical Congress, Boston Meeting (advance print), pp. 1–5, Cambridge, 1909.

Ishikawa, C.

'13 Einige Bemerkungen über den leuchtenden Tintenfisch, Watasea nov. gen. (Abraliopsis der Autoren) scintillans Berry, aus Japan. Zoologischer Anzeiger, Bd. 43, pp. 162–172, text fig. 1–6, December, 1913.

Joubin, L.

'93 Recherches sur l'appareil lumineux d'un céphalopode, *Histioteuthis ruppellii*, Verany. Bulletin Société Scientifique Médicale de l'Ouest, V. 2, pp. 49–80 [1–32], text fig. 1–10, 1893.

'93a Note complémentaire sur l'appareil lumineux d'un céphalopode: Histioteuthis Ruppellii, Verany. Bulletin Société Scientifique Médicale de l'Ouest, V. 2, pp. 161–169 [1–9], 1893.

'93b Note sur une adaptation particulière de certains chromatophores chez un céphalopode (l'oeil thermoscopique de Chiroteuthis-Bomplandi Vérany?). Bulletin Société Zoologique France, V. 18, pp. 146–151 [1–6], 1 text fig., 1893.

'93c Quelques organes colorés de la peau chez deux céphalopodes du genre *Chiroteuthis*. Mémoires Société Zoologique France, V. 6, pp. 331–343 [1–13], text fig. 1–12, 1893.

'94 Nouvelles recherches sur l'appareil lumineux des céphalopodes du genre *Histioteuthis*. Bulletin Société Scientifique Médicale de l'Ouest, V. 3, pp. [1–15], text fig. 1–7, 1894.

'95 Note sur les appareils photogènes cutanés de deux céphalopodes: *Histiopsis atlantica* Hoyle et *Abralia oweni* (Verany) Hoyle. Mémoires Societe Zoologique France, V. 8, pp. 212–228 [1–17], text fig. 1–11, 1895.

'05 Note sur les organes lumineux de deux céphalopodes. Bulletin Société Zoologique France, V. 30, pp. 64–69, text fig. 1–2, 1905.

'05a Note sur les organes photogènes de l'oeil de *Leachia cyclura*. Bulletin Musée Océanographique Monaco, No. 33, pp. 1–13, text fig. 1–7, April, 1905.

'12 Etudes préliminaires sur les céphalopodes recuellis au cours des croisières de S. A. S. le Prince de Monaco. 1re Note: Melanoteuthis lucens nov. gen. et sp. Bulletin Institut Océanographique Monaco, No. 220, pp. 1–14, text fig. 1–12, January, 1912.

'12a Sur les céphalopodes capturés en 1911 par S. A. S. le Prince de Monaco. Comptes Rendus Séances Academie Science, Paris, V. 154, pp. 395–397 [1–3], 1912.

Meyer, W. T.

'06 Über das Leuchtorgan der Sepiolini. Zoologischer Anzeiger, Bd. 30, pp. 388–392, text fig. 1–3, July, 1906.

'08 Über das Leuchtorgan der Sepiolini: II., Das Leuchtorgan von Heteroteuthis. Zoologischer Anzeiger, Bd. 32, pp. 505–508, text fig. 1–4, January, 1908.

Naef, A.

'12 Teuthologische Notizen, 1–2. Zoologischer Anzeiger, Bd. 39, pp. 241–248, March, 1912.

Sasaki, M.

'12 Hotaru-ika. Toyama-ken, Sui-san Kumiai Hokoku, pp. 1–29, 2 pls. and chart, 1912. (In Japanese.)

'13 Hotaru-ika. Zoölogical Magazine, Tokyo, V. 25, pp. 581–590, pl., 1913. (In Japanese.)

'14 Observations on Hotaru-ika Watasenia scintillans. Journal College Agriculture, Tohoku Imperial University, V. 6, pp. 75–107, 1 text pl., pl. 1–3, November, 1913.

Tryon, G. W., Jr.

'79 Cephalopoda. Manual Conchology, V., 1, pp. 1–316, pl. 1–112, Philadelphia, 1879.

Vérany, J. B.

'51 Mollusques méditerranéens, observés, décrits, figurés et chromolithographiés d'apres le vivant, I., Céphalopodes de la Méditerranée. Gênes, 1851.

Vivanti, A.

'14 Contributo alla conoscenza dei Cefalopodi abissali del Mediterraneo. Ricerche sulla Carybditeuthis maculata n. g. n. sp. dello Stretto di Messina. Archivio Zoölogico Italiano, V. 7, pp. 55–79, text fig. 1, pl. 3–5, 1914.

Watasé, S.

'05 Luminous Organs of Abraliopsis, a New Phosphorescent Cephalopod from the Japan Sea. Zoölogical Magazine, Tokyo, V. 17, pp. 119–122, 1 text fig., June, 1905. (In Japanese.)

RAPIDITY OF ACTIVATION IN THE FERTILIZATION OF NEREIS.

H. B. GOODRICH.

The following experiments were designed as a test of the rapidity of the action of the spermatozoön of *Nereis* in relation to the initiation of the processes of maturation. The almost instantaneous effect of the contact of the spermatozoön in stimulating the formation of the fertilization membrane and, in this case, of the jelly is well known. It is also usually observed that, in the case of those eggs in which maturation follows insemination, that polar-body formation will occur without fail even if later observation shows that no cleavage follows. It might be conceived that the continued action of the spermatozoön were necessary to cause maturation. The results outlined below indicate that maturation in *Nereis* follows almost, if not quite as brief an application of the stimulus as is necessary to initiate the jelly formation.

The experiments of Lillie ('11) on *Nereis limbata* showed clearly that the removal of the spermatozoön later than twenty-one minutes after insemination by a process of centrifuging did not interfere with the maturation of the egg. It was, however, found impossible by this method to remove the sperm earlier than twenty-one minutes after fertilization. Various workers have suggested (I am indebted to Dr. Chambers for first calling this to my attention) that the Barbour apparatus for micro-dissection offered a means of removal during this earlier period. The manipulation of the instrument for this purpose has proved most successful. The spermatozoön may readily be removed shortly after attachment and with more difficulty later because of the increasing strength of adherence to the egg and the viscidity of the head of the spermatozoön. The viscidity is shown in attempts to remove the spermatozoön at about 35 minutes after insemination. The head, remaining attached in the region of the perforatorium may be extended by

the needle some five or ten times its original length. In these later stages the method of removal by centrifuging is more practical.

The experiments were made during the summer of 1919 at the Marine Biological Laboratory at Woods Hole, Mass. Sperm and eggs were obtained from animals caught the evening before and kept in the upper compartment of the refrigerator during the night. Sperm dilutions of about 1/400 to 1/500 were used for most of the experiments. This dilution is not such as will insure that only one spermatozoön will come in contact with each egg (Lillie, '15), but it is sufficiently dilute so that polyspermy rarely results. Polyspermy was noted in a few of the eggs in experiments 6 and 7. The eggs were placed in a dilute suspension of chinese ink, at once fertilized and transferred to the coverslip which forms the roof of the moist chamber. The coverslips had been previously carefully cleaned of all traces of oil or grease in order to allow the drop of water to spread, thus forming a thin film which compresses the eggs slightly as is necessary in order to hold the egg firmly during the operation. The apparatus was always in complete readiness for the operation and it was usually possible to locate promptly an attached spermatozoön and to carefully push or rub it from its point of attachment to the egg. Spermatozoa remained immobile after detachment. Two or three operations were usually carried out on each coverslip. More were not attempted because the delay would allow too great an evaporation of water in the necessarily imperfect moist chamber, thus causing greater compression of the egg and also greater concentration of the sea water. The position of the operated eggs among others was plotted, the coverslip removed and placed under a binocular microscope, the selected eggs were isolated and removed to separate dishes for observation. Frequently the remainder of the eggs on the cover slip was also removed for cleavage counts (Control 2). These were the last to be removed and in some cases had by that time undergone considerable compression (in other cases the cover slip was slightly flooded to prevent this). This may in part account for the low percentage cleavage recorded frequently under Control 2.

The eggs were then kept under observation to note the forma-

tion of the polar bodies and in the second series (Table II.) to note possible cleavage. In all but two of the sixty eggs the polar bodies formed. I am inclined to think that as these two were among the earlier ones observed in this case the negative record may be due to faulty observation. Also in only three cases and one of these from a clearly polyspermic lot of eggs was cleavage observed. The time of removal varied from 1¾ minutes to 13½ minutes after insemination. It is impracticable to remove the sperm in most cases earlier than two minutes after insemination as it is difficult to discriminate between spermatozoa lying against the egg and those that have effected an attachment. The average of elapsed time from insemination to removal was 6.2 minutes and in sixteen cases the spermatozoön was removed in less than 4 minutes after attachment, and in five cases in less than 3 minutes. It seems therefore clear that in so far as maturation is concerned, the full stimulating effect of the spermatozoön is effective within a very few minutes after attachment and quite possibly it is only a matter of seconds.

The results clearly support the concept that the first phase of activation of the egg-membrane formation and maturation is initiated by the spermatozoön which "activates a substance, or ferment-like bodies, contained within the egg" (Lillie, '19, p. 159), rather than by the continuous introduction of some lysin-like substance through the slender perforatorium. For it seems improbable that the spermatozoön could introduce in less than two minutes through the perforatorium which has a cross section area of perhaps one ten-millionth of the surface area of the egg, a sufficient amount of material to take part in reactions throughout the egg. It should, however, be noted that under certain conditions (heating, Just, '15) it is possible to initiate jelly formation without maturation following. This may be taken to indicate that a lesser stimulus gives a lesser result and possibly if it were practicable to remove the sperm more promptly that jelly formation only would result. On the other hand in the cited case we may be dealing with a stimulus differing in kind rather than in degree. It should also be noted that these results are not at variance with the concept that cortical changes form an all important intermediate step in the activation of the egg.

In some cases (Table II.) it was possible to note the time of formation of the first polar body. The average of elapsed time after insemination was 35.8 minutes and no correlation was noted between the duration of attachment of the sperm and the time of formation of the polar bodies.

TABLE I.

Date.	Experiment Number.	Time of Fertilization.	Egg.	Time of Removal of Sperm.	Minutes Elapsed.	Polar Body Formation.	Cleavage.	Controls.	
								No. 1.	No. 2.
July									
17	1	10.48	A	10.56	8	+	−	+	60%
			B	11.02	14	+	−	+	"
	2	2.44	A	2.54	10	+	−[1]	+	90%
			B	2.58	14	+	−[1]	+	"
18	3	5.29	A	5.32½	3½	−	−[1]	+	65%
			B	5.37½	8½	+	−[1]	+	"
19	4	9.59	A	10.03	4	−	−[1]	+	70%
			B	10.08½	9½	+	−[1]	+	"
			C	10.10	11	+	−[1]	+	"
20	5	2.29½	A	2.33½	4	+	−	+	60%
	6	2.40	B	2.51	11	+	−[2]	+	"
21	7	9.40½	A	9.48	7½	+	+[2]	+	90%
	8	10.14½	A	10.18½	4	+	+	+	40%
			B	10.21¾	7¼	+	+	+	"
	9	11.26	A	11.27¾	1¾	+	−[1]	+	50%
			B	11.32	6	+	−[1]	+	"
	10	2.54½	A	3.00	5½	+	−	+	70%
			B	3.03	8½	+	−	+	"
	11	3.13	A	3.16½	3½	+	−[1]	+	
			B	3.21½	8½	+	−[1]	+	

As noted in Table I. some eggs were fixed at about time of the first cleavage with view to study of cytological changes. Too few of these have survived the ordeal of embedding and sectioning to make any detailed study valuable. I noted that in some cases only chromosomal vesicles and in other cases well-formed chromosomes were present at the time of the first cleavage spindle. This may be compared with the observation of Lillie ('15) where chromosomes were formed in eggs from which sperm were removed by centrifuging but not in eggs which were caused to maturate by centrifuging without insemination.

The appended tables outline the experiments. Full data are

[1] Egg fixed at about time of expected first cleavage. No indication of cleavage at that time.

[2] Poly-spermy observed in same lot on cover-slip.

not present in all cases. The experiments were in part preliminary and the desirability of various controls and observation became apparent as they progressed. In as much as the work

TABLE II.

Date.	Experiment Number.	Time of Fertilization.	Egg.	Time of Removal of Sperm.	Minutes Elapsed.	Polar Body Formation.	Time Formed.	Minutes Elapsed.	Cleavage.	Controls. No. 1.	Controls. No. 2.	Controls. No. 3.
July												
23	12	9.47	A	9.50	3	+	10.19	33	−	+	60%	
			B	9.53½	6½	[3]				+		
			C	9.54½	7½	+	10.20	34	−	+	"	
	13	10.20	A	10.23¼	3¼	+	10.52	32	−	+	80%	
			B	10.25⅓	5⅓	+			−	+	"	
	14	11.05½	A	11.08½	3	+			−	+		
	15	11.36½	A	11.38½	2	+			−	+		
			B	11.42½	6	+			−	+		
			C	11.43½	7	+			−	+		
	16	2.40	A	2.48½	8½	+	3.21	31	−	+	90%	
			B	2.53½	13½	+	3.17	37	−	+	"	
	17	3.16½	A	3.24½	8	no. obs.			−[4]	+		
			B	3.24½	9	+	3.49½	33	−[4]	+		
			C	3.26½	10	+			−[4]	+		
			D	3.28	11½	+			−[4]	+		
24	18	10.15	A	10.18½	3½	+	10.46	31	−[4]	+		
			B	10.22	7	+	10.49	34	−[4]	+		
	19	10.51½	A	10.55	3½	+	11.22½	31	−[4]	+		
			B	10.57½	6	+			−[4]	+		
			C	10.58½	7	no obs.			−[4]	+		
	20	11.26	A	11.29	3	+	11.56½	30½	−[4]	+		
			B	11.33½	6½	+	11.57½	31½	−[4]	+		
	21	3.18½	A	3.21¾	3¼	+	3.53	35	−[4]	+		
			B	3.24	5½	+	3.53	35	−[4]	+		
			C	3.25	6½	+	3.52	34	−[4]	+		
25	22	9.33	A	9.36	3	+	10.05½	32½	−	+	60%	
			B	9.38	5	+	10.06	33	−	+	"	
			C	9.39	6	+	10.06	33	−	+	"	
	23	10.16	A	10.18½	2½	+			−	+	58%	
			B	10.21½	5½	+			−	+	"	
	24	10.48½	A	10.51½	3	+			−	+	57%	
			B	10.52	3½	+			−	+	"	
			C	10.55	6½	+			−	+	"	
			D	10.57	8½	+			−	+	"	
	25	11.34	A	11.37½	3½	+	12.15	41	−	+	45%	
			B	11.43½	9½	+			−	+	"	
	26	2.19¾	A	2.42½	4¾	+	2.57	37¼	−	+	25%	
	27	2.47	A	2.49	2	+	3.25	38	−	+	55%	+
	28	3.54½	A	3.57	2½	+	3.37	42½	−	+	80%	+
			B	4.00	5½	+	3.36	41½	−	+	80%	+
26	29	9.52	A	9.55	3	+	10.27	35	−	+	66%	+
	30	10.29	A	10.32¾	3¾	+	11.02	33	−	+	"	+
			B	10.35	6	+	11.03	34	−	+	57%	
	31	11.05½	A	11.07½	2½	+	11.38½	33	−	+	75%	+
			B	11.10¾	5¼	+	11.42½	37	−	+	"	+

[3] Egg disintegrated.

[4] No specific record in original notes of the absence of cleavage.

was stopped by the end of the *Nereis* "Run" and as the observations in regard to maturation were complete it seems to me to be best to present the work in its present form.

TABLES.

The headings are mostly self-explanatory. Under Polar Body Formation and Cleavage a plus sign (+) indicates that the process was observed and a minus (−) the reverse. Control No. 1 are eggs from the same lots as in the experiments fertilized a few minutes before the experiment began from the sperm of the same male used. Control 2 are eggs not operated on from the coverslip and Control 3 are eggs of the same inseminated lot mixed with chinese ink from which those placed on the cover slip were taken. The plus sign (+) under Controls 1 and 3 indicates the practically 100 per cent. cleavage usually realized with *Nereis* eggs.

WESLEYAN UNIVERSITY,
January, 1920.

LITERATURE CITED.

Just, E. E.

'15 Initiation of Development in *Nereis*. BIOL. BULL., Vol. 28.

Lillie, F. R.

'11 Studies of Fertilization in *Nereis*, I and II. Jour. Morph., Vol. 22.

'15 Studies of Fertilization, VII. BIOL. BULL., Vol. 28.

'19 Problems of Fertilization. University of Chicago Science Series.

IMMUNITY AND THE POWER OF DIGESTION.[1]

VERA DANCHAKOFF.

COLUMBIA UNIVERSITY.

The proteins are the fundamental chemical constituents of the living protoplasm in all our tissues and organs. But the cells of the tissues do not take up the proteins as such, they are known to live on the products of protein digestion, on their building stones, the various amino-acids. And by a long process of synthetic character the cells of a multicellular organism build up out of these building stones their own individual proteins of a highly specific structure.

The multicellular organism possesses special organs of digestion which successfully perform the work of splitting the ingested food to the stage of amino-acids, in which stage they become capable of passing through the intestinal wall; they are absorbed here and transported to all our tissues, which in this manner are provided with various substances for their synthetic work in order to replace the wear and tear of the living substance during adult life or to build up organs and tissues during developmental stages.

Now are the cells of all our organs and tissues which, under usual conditions, are supplied for their synthetic work with ready made building stones, without one of the most characteristic and important properties of the living matter, viz., the power of splitting the chemically more complex proteins to the stage of amino-acids? Or are these cells not manifesting a digestive potency inherent in them, because they have no chance of manifesting it, because the ingested food does not reach them primarily and undergoes the hydrolytic changes before it is capable of being absorbed? Is it a lack of power or a lack of opportunity which prevents the cells of our tissues from manifesting a digestive capacity under usual conditions?

[1] Given at the meeting of the Section of Biology, New York Academy of Sciences, October 13, 1919.

A most wonderful property exhibited by the tissues themselves is to inhibit growth of foreign tissue. This resistance of the organism against proliferation of any heterogeneous cellular elements, be it normal tissues, tumor cells or microörganisms, is more generally known under the term of immunity. There is little need to emphasize the importance of any new information concerning this problem. Disregarding, however, the most earnest efforts and attempts to throw upon this property light sufficient to master it, no definitive knowledge has yet been acquired concerning the nature of this property.

In all organisms, plants and animals as well, the power of resistance against proliferation of any foreign tissue does not seem to depend upon the activity of a special organ in the organism. A graft of heterogeneous tissue simply does not take, a tumor graft does not grow, microörganisms do not proliferate, die and disappear in an immune or immunized animal. A living cell endowed with the faculty of proliferation, for example, a tumor cell, well known for its high rate of proliferation and on account of this property disastrous to the organism, on which it has settled, if grafted on an immune organism, will certainly find here all of the elements needed for the building up of its own protoplasm, for we know that the tissues of the organism are abundantly provided with various amino-acids, and these not being specific, can be used equally well by any tissue. And still a tumor cell will not live on an immune organism. It dies and disappears.

If now we inquire into the relation between the digestive power of the cell and resistance in a unicellular organism, we will see that both phenomena in this case are very closely connected. A unicellular organism can ingest another living organism, bacteria as well and the latter, while within a cytoplasmic vacuole of the former, are killed and consecutively digested by the enzymes of the phagocyte. The unicellular organism in this case proves to be immune against a possible intracytoplasmic proliferation of the ingested living individual, primarily and solely because of its digestive capacity.

The question whether in a multicellular organism any relation exists between its resistance against proliferation of a foreign tissue in it and a digestive power of its tissues is obscured by the

fact that the tissues of the organism do not exercise under normal conditions any digestive power, and it is not definitely settled, which of the various tissues, if any, besides those connected with the digestive tract, are endowed with a digestive power. And still there exist sufficient indications that the tissues of a multicellular organism, besides those highly specialized of the digestive tract, are equipped with enzymes which confer on them a digestive power similar to that so characteristic of the special elements in the digestive tract. If the tissues of a multicellular organism be put under the stress of starvation, under which condition no amino-acids will reach them from the absorbing surface of the digestive tract, the tissues will in this case use up tissue proteins, they themselves hydrolyze the proteins or digest them, which work is made possible by the presence of enzymes in these cells. And another example of digestive work performed by tissue cells of a multicellular organism can be found in the growth of tissue on artificial media. Here again the transplanted bits of tissue do not find within the culture medium ready building stones for the building up of their protoplasm in the form of amino-acids, but only proteins of the blood plasma clot. Here again the cells of the tissues themselves have a chance of performing the splitting or digestion of the proteins, and they do it. In both these cases we base our conclusions of the presence of enzymes in the tissue cells on the results of the experiments: on the continuation of the output of nitrogen by the organism during a diet free from nitrogen in the starvation experiment, and on the further growth of the tissue in the culture experiment. The fact only not the mechanism of the digestive activity by tissue cells is determined in both cases.

Some information concerning the mechanism, or rather concerning one of the mechanisms, by which cells of our body may digest, *i.e.*, split and assimilate protein may be gained by microscopical study. And this study informs us that in embryos developing from mesoblastic eggs, *i.e.*, from eggs containing great quantities of yolk, as for example, from birds eggs, all of the tissues contain at an early stage a great quantity of yolk granules in their cytoplasm, which are digested intracellularly and assimilated. Endodermal cells as well as ectoderm and mesoderm cells, even primitive germ cells, all are endowed with

an intracellular digestive capacity. And this capacity is surely not lost during the development of the embryo, at least the mesenchymal cells continue to exercise this faculty, and wherever cells in the developing organism have lost their normal correlation with other tissues, as for example, red blood corpuscles during the rearrangement of vessels, mesenchymal cells are seen to ingest them and digest them within their cytoplasm. The mechanism of this digestive activity is analogous to that observed in unicellular organisms. Enzymes are not given off as in the digestive tract, they work within the cytoplasm, they are endoenzymes. To a digestive activity of the mesenchymal cells is due the disappearance of aseptic emboli, trombi and infarcts; small sequesters may be entirely resorbed and catgut sutures disappear in the organism in a short time.

An intracellular digestive activity is exhibited in an intense way by the tissues of embryos developing from mesoblastic eggs in the early stages of embryonic development and only occasionally during adult condition. This power is dormant in the tissues of mammals during embryonic development, the embryo receiving all the materials needed for its development in the form of amino-acids from the maternal placenta, but is awakened at the first opportunity of being confronted with unsplit protein.

The digestive power of the tissues is well evident in respect to cellular protein in the dead form. Only in a unicellular organism did we see that their digestive activity confers on them a power over living organisms and cells as well and therefore becomes the source and the cause of their immunity against a possible proliferation of the ingested organisms. May the digestive capacity of the tissues of a multicellular organism, of which we have seen a few examples, be also in some way connected with the failure of a heterogeneous graft to take? Is this power not the deleterious factor which cannot be overcome by the grafted heterogeneous tissue and which inhibits its growth and proliferation?

The results of a long series of experiments, which I am going to illustrate, show without any doubt that at least in some cases it is the digestive activity of an adult mesenchymal cell which inhibits the growth of a heterogeneous tumor or rather destroys the actively growing tumor. The experiments were made with

different tumors, but only the Ehrlich sarcoma, and the tumor known at the Crocker Fund Laboratory, under the number 180, gave demonstrable results. It is known that mammalian tumors may be grown easily on chick embryos, but not on adult animals. Also normal adult chick tissue grows well on embryos of the same species. Therefore, embryonic tissues, more particularly the chick allantois, have been used by me as a culture medium, to bring together mammalian tumors and various adult chick tissues. It was expected that the study of the interaction of the tissues of the adult naturally immune fowl and of the mammalian tumor cells grown in a culture medium, for both equally favorable, might show whether the digestive capacity of the tissues more particularly that of the adult splenic mesenchymal cells may in any way be connected with the resistance offered by the adult animal to the grafting of the tumor on it. The experiments have shown that the Ehrlich sarcoma gives invariably a good growth if grafted alone, but if grafted in a mixture with the spleen, disappears even after a short period of proliferation. The short-lived fame of the small lymphocytes thought to be responsible for this disappearance is still in our memory. Only an absolute disregard of microscopical findings can explain how microscopical pictures similar to those shown here (Figs. 1, 2, 3 and 4), have been overlooked and how the small lymphocyte could become the fetish of the immunity.

Microscopical preparations, as seen in retouched photographs, which accompany this paper, illustrate in a striking way the process of disappearance of tumor foci surrounded by the adult splenic mesenchyme in the allantois. Two lines of activity are observed in the mesenchyme, it splits off numerous mobile cells of hemoblastic nature which differentiate further into granular leucocytes. This developmental potency is exhibited in an even more intensive way by the splenic adult mesenchyme, if the splenic tissue is grafted alone on the chick allantois. But the fact of grafting the splenic tissue together with tumor reveals in it a new potency and this is its power of isolating and surrounding tumor cells, of enclosing them in vacuoles and of digesting them within these vacuoles (Figs. 1, 2, 3 and 4). The adult splenic mesenchymal cell is apparently attracted toward the mammalian tumor cell. Contrary to the embryonic mesen-

chyme, the adult mesenchymal cells, once close to the tumor cell, will not indifferently pass by, but together with other mesenchymal cells, will tightly surround it. The tumor cell, in response to the approach of the adult mesenchymal cell, withdraws its cytoplasmic processes, becomes immobile and assumes soon a spherical shape. The Figure 1 illustrates a tumor focus in which the cells still intensely proliferate in the center as may be seen from numerous mitoses present. The tumor focus is however surrounded by a zone of mesenchymal syncytium of splenic origin. The cells of this tissue encircle the tumor cells at first surrounding them very tightly (Figs. 1 and 2, *x*). The adult splenic mesenchymal cells of the fowl treat a heterogeneous mammalian tumor cell, which, if present alone in the embryonic allantois, would live and proliferate in no other manner than if it were a block of dead protein. They gather around the tumor cells and enclose them into a capsule and then secrete a fluid within the little cavity occupied by the tumor cell. The nature of this fluid is such that a disintegration of the tumor cell takes place and a complete splitting of its proteins, it must therefore contain proteolytic enzymes. The tumor cell gradually loses its structure (*Tc'*, Figs. 1, 2, 3 and 4) is transformed into a block of structureless protein (*Tc''*, Figs. 1, 2, 3 and 4) and finally disappears completely. Tumor cell after tumor cell is digested in this manner. In the case of the Ehrlich sarcoma, the rate of digestion of the tumor cells by the splenic mesenchyme is higher than the rate of proliferation of the tumor cells, and the grafted tumor, even after a good start of development, disappears. The same process is observed in relation to the sarcoma 180. Only in this case the rate of proliferation of these tumor cells is higher than the rate of their digestion by the splenic mesenchyme, and the tumor still grows in spite of the existence of a peripheral zone around it, in which the process of digestion of tumor cells by mesenchymal cells is most evident.

The study of microscopical preparations allows us to follow the gradual changes which the tumor cells undergo within the vacuoles surrounded by mesenchymal cells, changes which lead to the full disappearance of the tumor cells. Figures 2, 3 and 4 show very clearly how healthy tumor cells *H Tc* are cut off from the tumor focus, the tumor cells withdrawing their processes

and rounding up, how the tumor cells gradually lose their structure *Tc'*, their cytoplasm becoming vacuolized, their nuclei pycnotic and how finally the tumor cells disappear entirely.

Now in respect to the small lymphocytes, may they not be still connected in some indirect way with the disappearance of the tumor cells? Round-cell infiltration has been described around the disappearing tumor grafts within the allantois. But though the small lymphocyte is a round cell, not every round cell is a small lymphocyte. Round cell infiltration exists indeed around such grafts. This infiltration, however, at a closer study proves to consist not of small lymphocytes, but of round, mobile cells of hemoblastic nature which, as mentioned above, differentiate into granular leucocytes. As has been shown in one of my previous papers, the small lymphocytes are not very viable in the allantois and in most cases quickly disappear in the grafted splenic tissue.

It is a positive tropism between the adult mesenchymal cell and the tumor cell expressed in the phagocytic power of the mesenchymal cell over the tumor cell and the digestive activity of the adult mesenchymal cell which prove to be the direct factors in the disappearance of the tumor cells in a double mixed graft of tumor and spleen. One of the remarkable results of the various experiments undertaken in this direction is the fact, that only the adult splenic mesenchyme, not the embryonic, exhibits the capacity of digesting tumor cells. This observation well corresponds with the fact that the embryo fails to resist growth of heterogeneous tissue.

I have already mentioned that only in respect to the Ehrlich sarcoma and to the sarcoma 180 could I obtain results which clearly demonstrate the phagocytic and digestive activity of the adult splenic mesenchymal cells. The study of the growth of other tumors and of the check of their growth is sufficiently advanced to enable me to conclude that the inhibition of the carcinoma growth is effected by a mechanism not altogether identical to that described in relation to the Ehrlich sarcoma cells. Must this fact astonish us? I do not think so. To expect a literally identical response of the mesenchymal cells to different agents would be just as inconsistent as to expect the digestive activity in all multicellular animals to proceed in a perfectly identical

manner. And we know that this is not the case. We know that in multicellular organisms there exists a digestive cavity, into which free enzymes are poured, we know, however, that in sea-anemones, though a digestive cavity does exist, no ferments had ever been discovered in it and the digestion of the food proceeds through the immediate apposition to it of definite cellular elements.

The fact that at least in some cases the phagocytic and digestive activity of the mesenchymal adult cells is found to be the deleterious factor, which cannot be overcome by some of the tumors and which inhibits the growth of these tumors, or rather destroys an actively growing tumor, throws a new light on the immunity problem in general, since the power of resistance against proliferation of foreign tissue, though manifested in different ways, can certainly not be of a fundamentally different nature.

EXPLANATION OF FIGURES.

The figures are retouched photographs from original preparations. The Figure 1 at about 400, the Figure 3, 700 and the Figures 2 and 4, 1000 diameters.

Abbreviations.

Gr Lc. Granular leucocytes.
H Tc. Healthy tumor cells.
S Ms. Splenic mesenchyme exercising a phagocytic and digestive activity.
Tc'. Tumor cells in various stages of disintegration.
Tc''. Tumor cells transformed into blocks of almost structureless protein.
V. Vessel.
X. Tumor cells tightly surrounded by mesenchymal cells.

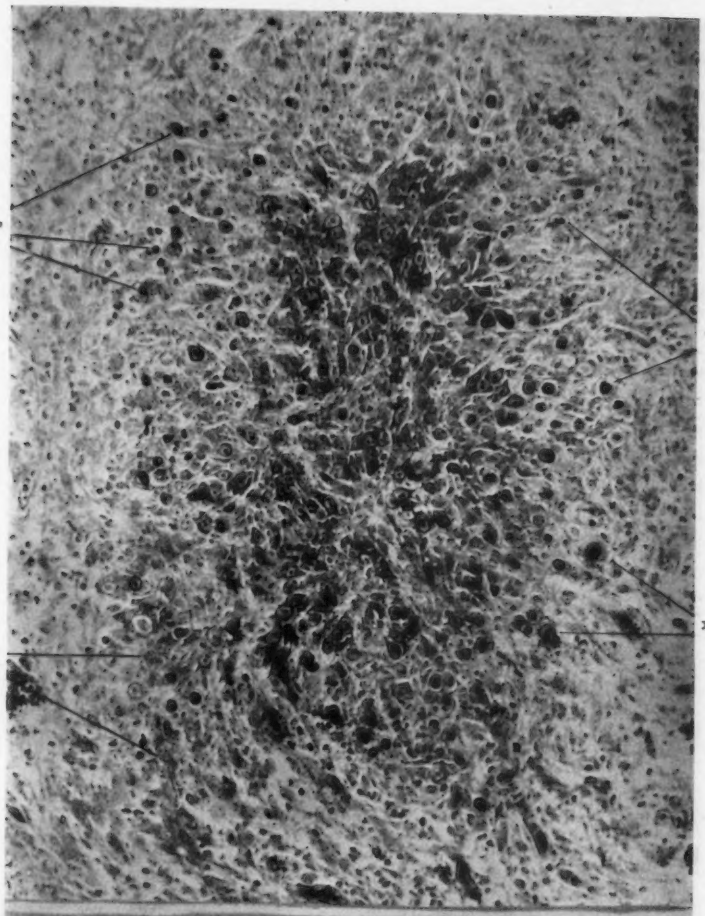
The figures illustrate the result of growth of mixed grafts, consisting of mammalian tumor and adult chick spleen on the allantois.

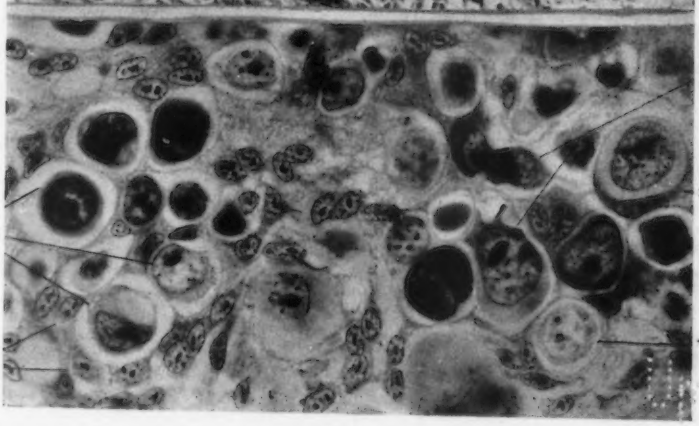
Plate I.

Fig. 1. Actively growing tumor focus of 4 days growth in the allantois. In the center groups of healthy tumor cells with numerous mitoses. The focus of healthy tumor tissue is surrounded by a zone in which the splenic mesenchymal cells encircle the tumor cells and digest them in closed vacuoles.

Fig. 2. Small area of the peripheral zone around a tumor focus of 4 days growth. Only two tumor cells (*H Tc*) still exhibit a healthy structure. All the others (*Tc'* and *Tc''*) show various changes dependent upon the digestive activity of the splenic mesenchymal syncytium enclosing them in vacuoles.

FIG .1.





V. DANCHAKOFF.

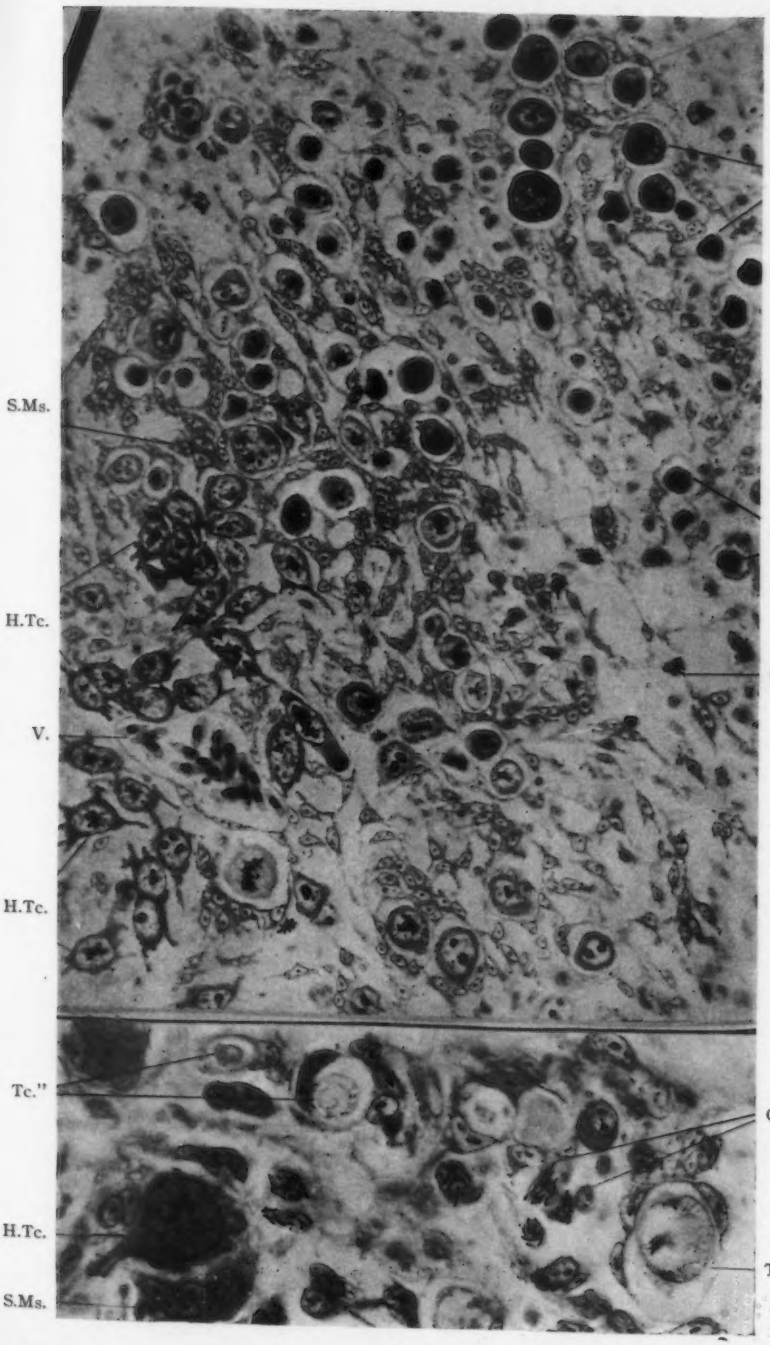
FIG. 2.

PLATE II.

FIG. 3. Small region of the peripheral zone around a tumor focus of 4 days growth, showing a group of healthy tumor cells (*H Tc*) and numerous other tumor cells in various stages of disintegration.

FIG. 4. Small area of the peripheral zone around a tumor focus of 4 days growth, illustrating the disintegration of the tumor cells within the vacuoles, formed by the splenic mesenchymal cells.

FIG. 3.



V. DANCHAKOFF.

FIG. 4.

CHROMOSOME STUDIES IN TETTIGIDÆ. II. CHROMOSOMES OF PARATETTIX BB AND CC AND THEIR HYBRID BC.[1]

MARY T. HARMAN.

Introduction 213
Observations 214
1. Spermatogonial Divisions 214
a. Chromosomes of CC 215
b. Chromosomes of BB 215
c. Chromosomes of BC 216
2. Growth Period 216
3. Spermatocyte Divisions 217
4. The X-chromosome 218
Discussion 218
1. Synapsis and Heredity 219
2. Chromosomes in Hybrids 221
Conclusions 222
Literature Cited 223
Explanation of Plates 226

INTRODUCTION.

As was stated in a previous paper (Harman '15) the work on the cytological constitution of the germ-cells of *Paratettix* has been undertaken for the purpose of discovering whether or not the microscope will reveal any differences in the germ-cells of very closely related forms which may be correlated with the differences in hereditary characteristics. Three forms have been considered in this paper; two breeding true to type, BB and CC and their hybrid BC (Nabours '14 and '17).

In his breeding work with *Paratettix* Nabours ('17) has considered only the color patterns of the pronota and femora of the jumping legs. He has found fourteen distinct color patterns which behave as a unit and cannot further be broken up. These color patterns he designated as multiple allelomorphs. In most crossings between different pure types the resultant hybrid is readily discernible from either parent. In those hybrids which

[1] Contribution from the Zoölogical Laboratory, Kansas State Agricultural College, No. 27.

are not so readily discerned from the parents, a closer examination shows that the pigment of each parent is distributed in about equal proportions. There is no evidence of dominance nor crossing over, and the proportions of offspring are almost perfectly Mendelian even in small numbers.

It is from these grouse locusts that the material for the present paper was obtained. The author wishes again to thank Dr. R. K. Nabours for this material and express her appreciation to him for furnishing her the material at a time when she could use it and for giving her access to his records from which she has been able to know the pedigrees of her animals.

Observations.

Only the male germ-cells have been studied. Observations have been made upon the spermatogonial divisions, the growth period, synapsis and the maturation divisions. Special consideration has been given to the metaphase plate, polar view, of the spermatogonial divisions, the behavior of the chromatin material during the growth period and the manner of the formation of the first spermatocyte chromosomes.

1. *Spermatogonial Divisions.*

In all three forms the thirteen spermatogonial chromosomes in the metaphase are arranged on the spindle, having the appearance of the spokes on the hub of a wheel (Fig. 1). As may be seen in a metaphase plate, polar view (Figs. 3 to 12), these chromosomes are in pairs and the members of each pair are approximately of equal size and of similar shape. The accessory chromosome is larger than the two smallest pairs and smaller than the other chromosomes. However, many times the difference in size between the chromosomes of the second and third pairs and the X-chromosome is so slight in the spermatogonia that it is difficult to distinguish them. In Fig. 9, the homologous chromosomes of the different pairs are designated by the same number.

In the anaphase the chromosomes take a position more in the same direction of the fibers of the spindle rather than at right angles to it as in the metaphase (Fig. 2). The change from the

perpendicular position in the metaphase to the parallel position in the anaphase would indicate a telomitic fiber attachment. In the late anaphase and early telophase the chromatin material begins to take on a granular appearance before the chromosomes have formed into a spireme (Fig. 13). In fact the cell has completed its division before there has been much reconstruction of the chromatin material.

(*a*) *Chromosomes of CC.*—Figures 3, 4 and 5 are metaphase plates, polar view, of CC spermatogonial cells. Four of the thirteen chromosomes are decidedly larger than the others. The six smallest chromosomes are somewhat narrower at the proximal end than at the distal end. The third pair, marked 3 in these figures, are distinctly pointed at the proximal end, but there is no indication of a bend or hook on this pointed end. The X-chromosome is more ovoid and when lying in certain positions shows a slight constriction in the middle. Figure 3 shows one of the larger chromosomes split at its distal end. This must be a precocious division, as it was not observed in any of the hundreds of other cells examined. Often one of the chromosomes is observed in the center of the spindle (Fig. 4), or the proximal end may be near the center (Fig. 5).

(*b*) *Chromosomes of BB.*—In general the arrangement of the chromosomes of BB are like those of CC (Figs. 9–12). There are the two large pairs, the three smaller pairs and an intermediate pair. The X-chromosome is ovoid with a slight constriction near the middle (Fig. 9). There also occurs the crowding of the chromosomes toward the center of the spindle. The most evident difference between BB and CC is in the third pair, marked 3 in all the figures. Not only are these chromosomes pointed at their proximal ends, but the point is considerably bent to form a definite hook in BB. Sometimes this hook forms an acute angle with the other part of the chromosome (Fig. 12), but more often the bend is rounded (Fig. 10). The hooked chromosome on the left in figure 10 is much smaller than its homologue on the right, but this is due to the fact that a portion of its distal end was cut off as was also the large chromosomes on either side of it. These parts of chromosomes were found in the preceding section.

(*c*) *Chromosomes of BC.*—The hybrid BC (Figs. 6–8) has only one hooked chromosome, while its homologue is merely pointed like in CC. This difference is constant in the many metaphase plates examined. There is some variation in the shape of the chromosomes and the size of the bend of the pointed end. In Fig. 6 the hooked member of the third pair is almost crescent shaped, while in the other cells the hooked chromosome is thick at the distal end, tapers toward the proximal end, and then bends abruptly.

2. Growth Period.

Figures 13 to 24 illustrate the changes which take place in the growth period. At the end of the last spermatogonial divisions the chromosomes have a crinkled appearance. Figure 13 shows all thirteen of the chromosomes in this condition. However the X-chromosome is more compact than the others. This crinkled appearance is due to the chromatin becoming less compact and more granular. There is no sign of a nuclear membrane nor any indication of a separation of the nuclear area from the cytoplasm. Soon the chromatin becomes very finely granular and the chromosomes unite end to end (Fig. 14). Shortly after this stage the chromatin has the appearance of a finely granular continuous thread and a light area separates the cytoplasm from the nuclear material (Figs. 15 and 16). The cell begins to increase in size and the chromatin thread becomes contracted into a ball (Fig. 17). There seems to be no definite polarization to the loops of the spireme, but it has more the appearance of a tangled mass. Sometimes it is with difficulty that the individual fibres are distinguished. Now both the nucleus and the entire cell increase greatly in size (Figs. 18–21) and the chromatin material forms into a thicker thread (Fig. 18). Then the chromatin thread becomes looser and the granules are more concentrated, particularly in places throughout the strand (Fig. 19). The portion of chromatin marked X never becomes as granular as the remainder of it. This chromatin is the sex-chromosome and can be identified in all the stages.

Following the opening up of the tangled knot the chromatin thread breaks into pieces (Figs. 20 and 21). Figure 22 shows thirteen pieces, twelve of which are uniting in pairs by an end

to end union. Figures 23 and 24 show successive stages in the formation of the primary spermatocyte chromosomes. As the chromosomes become more compact in appearance the nuclear membrane disappears and there is no definite boundary again between the nucleus and cytoplasm (Fig. 24).

During all these changes there has not been the least appearance of a side by side pairing of the chromosomes, neither has there been any indication of a longitudinal splitting of the chromatin material either as chromosomes or as a chromatin thread. With the breaking up of the chromatin thread always more than the haploid number of chromatin elements may be seen, and in many instances there is evidently the diploid number. This surely justifies the conclusion that at the end of the growth period previous to the formation of the primary spermatocyte chromosomes there appears the diploid number of chromosomes and that the real pairing of the chromosomes does not take place in the spireme stage. It seems, also, very evident that the union of the chromosomes is by telosynapsis. As was previously stated (Harman '15), the dumb-bell shape of the chromosomes is not due to the manner of the division of the chromosomes, but is a result of the way they are formed. This shape is noticed in some of the chromosomes even before the entire chromatin thread is broken up (Fig. 21), and certainly before the first spermatocyte spindle is formed (Fig. 24).

3. *Spermatocyte Divisions.*

There are six dumb-bell shaped bivalent chromosomes and an ovoid univalent chromosome in the primary spermatocyte. These arrange themselves longitudinally on a large spindle (Figs. 25 and 26). The univalent, or X-chromosome, lies near the periphery of the spindle. The other chromosomes are crowded toward the center. Most often they are so closely crowded together that it is difficult to see them all at once in a lateral view of the spindle. Always one of the medium-sized chromosomes lies very near the second-largest chromosome (Figs. 26 and 30).

In the first division all the bivalent chromosomes divide at the constricted part of the dumb-bell (Fig. 27). In other words,

they have divided at the point of union as we have previously seen. As the result of this division the chromosomes of the anaphase are ovoid. The X-chromosome does not divide in this division but passes to one pole much in advance of the others. The first division then is truly a reductional division.

The spindle of the secondary spermatocyte is nearly spherical. Early in the metaphase the chromosomes show a longitudinal split (Figs. 28 and 29). All of the chromosomes divide in this division. It is an equational division in the sense of the dividing of the chromatin material of original chromosomes into halves.

4. *The X-Chromosome.*

The X-chromosome is a persistent portion of chromatin material which can easily be identified throughout the growth period. It never becomes finely granular, nor does it have the woolly appearance of the other chromatin elements. The other chromosomes lose their identity in the coiled thread, but the X-chromosome does not form a part of this coiled knot. With the formation of the primary spermatocyte chromosomes it may be distinguished from the others both by its shape and by its more compact appearance. On the primary spermatocyte spindle it is often found half way to the pole before the other chromosomes have completely divided. It is more difficult to recognize it in the secondary spermatocyte because all the chromosomes here are ovoid in shape, and the differences in size between some of the chromosomes are so much less. It behaves similarly to the other chromosomes here, neither being precocious in its division nor lagging.

Discussion.

Concerning many of the most obvious and essential points of the behavior of the chromatin in the maturation of the sperm, there is no longer any debate. It is agreed that during this period the number of chromosomes is reduced to one half. That this reduction in number is brought about by the union of chromosomes by pairs is also generally accepted. As to how this pairing has taken place has been the subject of much discussion, and upon the answer to this question depends the acceptance or rejection of other theories. It seems to the writer that when we

have accepted the thought that this reduction in number takes place by the union of chromosomes in pairs we have essentially also accepted the theory of the individuality of chromosomes. Then how do these individuals come together and finally separate again?

1. *Synapsis and Heredity.*

One can scarcely follow out the discussion of Janssen on *Batracoseps attenuatus*, and more especially when close attention is given to his drawings, without coming to the conclusion that with this form, at least, the chromosomes unite by parasynapsis and that it is perfectly possible in the twisting of certain chromatin elements to have an exchange of parts of homologous chromosomes, thus forming a convenient mechanism for the "crossing over" of hereditary characteristics if we accept the chromosome hypothesis of heredity. The "chiasmatypie" theory of Janssen ('09) furnishes a very convenient means for explaining some ratios in Mendelian inheritance.

An application of this chiasmatype theory, which should not pass without mention, is the work on *Drosophila*, particularly the work of Morgan and his students ('15). From the behavior of the hereditary characteristics a chromosome map has been constructed in which not only are the determiners of the characteristics located on definite chromosomes, but also the relative distances that these genes are from each other, is given. The position of the genes on the chromosomes is calculated from the percentages of cross-overs. In addition to the vast amount of genetic evidence for this condition in *Drosophila* there is also some cytological evidence.

Metz ('14) in dealing with five different types of chromosome groups of *Drosophila* shows that the chromosomes exhibit a close association in pairs at nearly all times and that before each cell division the pairs become so intimately associated that they may be said actually to conjugate. He further states that the union of the chromosomes is "unquestionably a side-by-side, or *parasynapsis* one." In a later paper (Metz '16) the same author in considering chromosome pairing in about eighty species of Diptera, many of which belong to the genus *Drosophila*, states that the pairing "certainly involves the essential features of a synaptic (parasynaptic) union."

Bridges ('17) confirmed the observations of Metz as to the spermatogonial and oögonial pairing, but deduced the kind of synapsis from his genetic data.

The genetic behavior of *Paratettix* is very different from that of *Drosophila*. In the hundreds of matings of *Paratettix* not a single instance of crossing-over has occurred. In accounting for the hereditary behavior of *Paratettix* without a knowledge of the cytological behavior Morgan has suggested the theory of identical loci. As has been previously shown, at no time during the growth period is there any indication of a double thread, and also there is evidence of an end to end union of the chromosomes to form the tangled thread of the contraction figure. Moreover at the end of the growth period the chromatin material forms the diploid number of chromatin elements. Also these chromatin elements agree in relative sizes to the spermatogonial chromosomes. Undoubtedly these parts unite end to end. In this case there is little chance for crossing-over, as only the ends of homologous chromosomes come in contact with one another. In view then of the cytological behavior, it seems more likely that the series of multiple allelomorphs may be accounted for by the kind of synapsis rather than by the theory of identical loci.

The writer is well aware that there is not perfect agreement as to how synapsis takes place in the Orthoptera, and even within a single family of the Orthoptera. McClung ('14) has recently reviewed the literature on the subject of synapsis in Orthoptera, and Wenrich ('16) has summarized the results. Briefly those who have described or assumed telosynapsis are: Montgomery ('05), *Syrbula;* Stevens ('05), *Stenopalmatus*, ('05) *Blatta*, ('10) *Forficula;* Wassilieff ('07), *Blatta;* Zweiger ('06), *Forficula;* Davis ('08), Acrididæ and Locustidæ; Buchner ('09), *Gryllus*, Œdipoda; Brunelli ('09), *Gryllus*, and ('10) *Tryxalis;* Sutton ('02, '03), *Brachystola;* Baumgartner ('04), *Gryllus*; McClung ('05, '08, '14), various Orthoptera; Nowlin ('08), *Melanoplus;* Pinney ('08), *Phrynotettix;* Robertson ('08), *Syrbula;* Carothers ('13), Acrididæ. Those who have assumed or described parasynapsis are: Gerard ('09), *Stenobothrus;* Morse ('09), Blattidæ; Stevens ('12), *Ceuthophilus;* Robertson ('15), Tettigidæ; Vejodvsky ('11–12), Locustidæ; and Otte ('07), Locusta. In addi-

tion to these Wenrich ('16) gives strong evidence for parasynapsis in *Phrynotettix* and ('17) *Chorthippus* and *Trimerotropis*.

May it not be that within the order Orthoptera, or even within the same family, that the kind of synapsis is different? The behavior in heredity is certainly different. The behavior of *Paratettix* in heredity has already been discussed. Bellamy ('17) has described a similar condition in the genus *Tettigidea*. He found five allelomorphic color patterns without any indication of cross-overs. The cytological constitution of the germ-cells of his material has not been examined. However, Robertson ('17) has described parasynapsis in *Tettigidea parvipennis*. Nabours ('19) has not only found crossing-over in *Apotettix*, but has also found parthenogenesis and crossing-over in those reproducing parthenogenetically. Certainly if we accept the chromosome hypothesis of heredity there must be a difference in the cytological behavior of the germ-cells of these genera. Certainly enough has already been said in this paper to show that the chromosomes pair by telosynapsis in *Paratettix*. With the many cross-overs in *Apotettix*, and the similar behavior in inheritance to *Drosophila*, one would expect the chromosome behavior to be different than in *Paratettix* and more like *Drosophila*. The writer has not yet studied the chromatin behavior in *Apotettix*.

2. *Chromosomes in Hybrids.*

In working with the chromosomes of hybrids Moenkhaus, Morris, Federley, Harrison and Doncaster, Pinney and others have found that despite the fact that the paternal chromosomes are in a foreign cytoplasm they retain their characteristic form, size and number. Moenkhaus ('04) pointed out that when *Fundulus* is crossed with *Menidia* two kinds of chromosomes are present in the fertilized egg and can readily be distinguished in later divisions, furthermore, that these two kinds of chromosomes are like the chromosomes of each parent respectively.

Morris ('14) found two types of chromosomes in the early cleavage stages of the hybrid of *Fundulus heteroclitus* ♀ and *Ctenolabrus adspersus* ♂. These types of chromosomes she identifies as the two types of parental chromosomes.

Pinney ('18) made a number of crosses with teleosts and also

made the reciprocal crosses. She found that some cytoplasm seemed to be more favorable to foreign sperm than others. In some instances a few chromosomes were eliminated, but those that did remain could be identified as to their paternal or maternal origin. Similar results were obtained by Federley ('13) and Harrison and Doncaster ('14) in hybrids of certain moths.

In the above cases cited there is no indication that these hybrids may occur in nature, and these offspring were obtained from animals which are accepted as distinct species. In the case of *Paratettix*, P. BB and P. CC are not yet admitted to be species by taxonomists, and they are known to cross in nature, judging from hybrids obtained in the wild and segregated in the laboratory. The chromosomes of each form are so very similar to those of the other forms in so many ways that it is only with close study that the differences are recognized. Yet, when once recognized it is found to be constant. The chromosomes of the hybrids of *Paratettix* are different from those of the fish mentioned above in that the parental chromosomes are more alike and that the cytoplasm of the egg seems to be perfectly compatible with the chromatin of the foreign sperm. They are different from those of the moths in that there are the same number of chromosomes in both forms and there is a complete synapsis with the homologous pairs of chromosomes.

Conclusions.

1. The third pair of spermatogonial chromosomes of BB are bent at the proximal end so as to form distinct hooks.

2. The third pair of spermatogonial chromosomes of CC are pointed at the proximal end, but there is no bend.

3. The third pair of spermatogonial chromosomes of the hybrid, BC, is composed of one hooked member and one pointed member.

4. During the growth period there is no indication of a parallel condition of the chromatin either in the chromatin thread or in the chromosomes.

5. At the end of the growth period there is evidently the diploid number of chromosomes formed which correspond in relative sizes to the respective spermatogonial chromosomes.

6. Synapsis does not take place in the thread but at the end

of the growth period by an end to end union of the homologous pairs of chromosomes.

7. The chromatin of the sex-chromosomes does not lose its identity during the growth period.

8. The first maturation division is a reductional division, and the second maturation division is equational.

9. The formation of the diploid number of chromosomes at the end of the growth period and the union of their homologous pairs by telosynapsis may explain the absence of any crossing-over in *Paratettix*.

LITERATURE CITED.

Baumgartner, W. J.

'04 Some New Evidence for the Individuality of Chromosomes. BIOL. BULL., Vol. 8.

Bellamy, A. W.

'17 Studies of Inheritance and Evolution in Orthoptera, IV. Multiple Allelomorphism and Inheritance of Color Patterns in Tettigidea. Jour. Gen., Vol. 7.

Bridges, Calvin B.

'17 Non-disjunction as a Proof of the Chromosome Theory of Heredity. Gen., Vol. 1.

Brunelli, G.

'09 La spermatogenesi del Gryllus desertus Pall. (Divisioni spermatogoniali e maturative.) Reale Academia dei Lincei, T. 2, serie 5a.

'10 La spermatogenesi della Tryxalis. Divisioni spermatogoniali. Memarie della Societa Italiana Scienze, T. 16, serie 3a.

Buchner, P.

'09 Das accessorische Chromosom in Spermatogenese und Ovogenese der Orthopteran, Zugleich ein Beitrag zur Kenntnis der Reduktion. Archiv. f. Zellforsh., Bd. 3.

Carothers, E. Eleanor.

'13 The Mendelian Ratio in Relation to Certain Orthopteran Chromosomes. Jour. Morph., Vol. 24.

Davis, H. S.

'08 Spermatogenesis in Acrididæ and Locustidæ. Bull. M. C. Z., Vol. 53.

Federley, H.

'13 Das Verhalten der Chromosomen bei der Spermatogénèse der Schmettalinge, etc. Zeit. Abst. Vererb., Bd. 9.

Gerard, P.

'09 Recherches la spermatogenese Chez Stenobothrus bigutulus (Linn.). Archives de Biologie, T. 24.

Harman, Mary T.

'15 Spermatogenesis in Paratettix. BIOL. BULL., Vol. 29.

Harrison, J. W. H., and Doncaster, L.

'14 On Hybrids between Moths of the Geometrid Subfamily Bistoninæ, with an Account of the Behavior of the Chromosomes in Gametogenesis in *Lycia* (*Biston*) *historia*, *Ithysia* (*Myssia*) *Zonaria* and in their Hybrids. Jour. Gen., Vol. 3.

Janssens, F. A.

'09 La théorie de la chiasmatypie Nouvelle interprétation des cinèses de maturation. La Cellule, T. 25.

McClung, C. E.

'05 The Chromosome Complex of Orthopteran Spermatocytes. BIOL. BULL., Vol. 9.

'08 The Spermatogenesis of *Xiphidium faciatum*. Kansas Univ. Sci. Bull., Vol. 4.

'14 A Comparative Study of Chromosomes in Orthopteran Spermatogenesis. Jour. Morph., Vol. 25.

Metz, Charles W.

'14 Chromosome Studies in Diptera I. A Preliminary Survey of Five Different Types of Chromosome Groups in the Genus *Drosophila*. Jour. Exp. Zool., Vol. 17.

'16 Chromosome Studies on the Diptera II. The Paired Association of Chromosomes in the Diptera and its Significance. Jour. Exp. Zool., Vol. 21.

Moenkhaus, Wm. J.

'04 The Development of the Hybrid between *Fundulus heteroclitus* and *Menidia notata* with Especial Reference to the Behavior of the Maternal and Paternal Chromatin. Am. Jour. Anat., Vol. 3.

Montgomery, T. H., Jr.

'05 The Spermatogenesis of *Syrbula* and *Sycosa*, with General Considerations upon Chromosome Reduction and the Heterochromosomes. Proc. Academy Nat. Sci. Philadelphia, February.

Morgan, T. H. and others.

'15 The Mechanism of Mendelian Heredity. Henry Holt and Co. New York.

Morris, Margaret.

'14 The Behavior of the Chromatin in Hybrids between *Fundulus* and *Ctenolabrus*. Jour. Exp. Zool., Vol. 16.

Morse, Max.

'09 The Nuclear Components of the Sex Cells of Four Species of Cockroaches. Archiv. f. Zellforsch., Bd. 3.

Nabours, Robert K.

'14 Studies of Inheritance and Evolution in Orthoptera I. Jour. Gen., Vol. 3.

'17 Studies of Inheritance and Evolution in Orthoptera II, III. Jour. Gen., Vol. 7.

'19 Parthenogenesis and Crossing-over in the Grouse Locust *Apotettix*. Amer. Nat., Vol. 53.

Otte, H.

'07 Samenreifung und Samenbildung bei Locusta viridissima. Zool. Jahrb. Abt. f. Anat., Bd. 24.

Pinney, Edith.

'08 Organization of the Chromosomes in *Phrynotettix magnus*. Kansas Univ. Sci. Bull., Vol. 4.

'18 A Study of the Relation of the Behavior of the Chromatin to Development and Heredity in Teleost Hybrids. Jour. Morph., Vol. 31.

Robertson, W. Rees Brebner.

'08 The Chromosome Complex of *Syrbula admirabilis*. Kansas Univ. Sci. Bull., Vol. 4.

'15 Inequalities and Deficiencies in Homologous Chromosomes; their Bearing upon Synapsis and the Loss of Unit Characters. Jour. Morph., Vol. 26.

'17 Chromosome Studies IV. A Deficient Supernumerary Accessory Chromosome in a Male of *Tettigidea parvipennis*. Kansas Univ. Sci. Bull., Vol. 10.

Stevens, N. M.

'05 Studies in Spermatogenesis with Especial Reference to the "Accessory Chromosome." Carnegie Inst. Publ. 36.

'10 An Unequal Pair of Heterochromosomes in *Forficula*. Jour. Exp. Zool., Vol. 8.

'12 Supernumerary Chromosomes and Synapsis in *Ceuthophilus*. BIOL. BULL., 22.

Sutton, W. S.

'02 On the Morphology of the Chromosome-group in *Brachystola magna*. BIOL. BULL., Vol. 4.

'08 The Chromosomes in Heredity. BIOL. BULL., Vol. 4.

Vejdovsky, F.

'12 Zum Problem der Vererbungsträger. Böhm. Gesell. wiss., Prag.

Wassilieff, A.

'07 Die Spermatogenese von *Blatta germanica*. Archiv. f. Mikr. Anat., Bd. 70.

Wenrich, D. H.

'16 The Spermatogenesis in *Phyrnotettix magnus*, with Special Reference to Synapsis and the Individuality of the Chromosomes. Bull. Mus. Comp. Zool., Vol. 60.

'17 Synapsis and Chromosome Organization in *Charthippus* (*Stenobothrus*) *curtipennis* and *Trimerotropis suffusa* (Orthoptera). Jour. Morph., Vol. 29.

Zweiger, H.

'06 Die Spermatogenese von *Forficula auricularia* L. Jena. Zeit., Bd. 42.

EXPLANATION OF PLATES.

All figures were made at table level by means of a Zeiss compensating ocular No. 6 and a 1.5 objective with the aid of a camera lucida. Figures 13, 14 and 15 were enlarged three and one half diameters. All others were enlarged two and one half diameters. All were reduced one third.

PLATE I.

FIG. 1. BC, metaphase plate, lateral view, of spermatogonial division.

Fig. 2. BC, late anaphase, spermatogonial division.

FIG. 3. CC, metaphase plate, polar view, spermatogonial division; X is the odd-chromosome, 3, 3 is the third pair of chromosomes, and 2 is one of the chromosomes of the fifth pair showing a precocious split.

FIGS. 4 AND 5. CC, metaphase plates, polar view, spermatogonial divisions; 3, 3 third pair of chromosomes.

FIGS. 6, 7 AND 8. BC, metaphase plates, polar view, spermatogonial divisions; 3, 3^1 third pair of chromosomes, 3^1 is the hooked chromosome of the third pair.

FIG. 9. BB, metaphase plate, polar view, spermatogonial division; the numbers 1, 1–2, 2, etc., to 6, 6 are homologous pairs of chromosomes, X is the odd-chromosome. It will be noted that the members of the third pair are both hooked chromosomes in BB.

FIGS. 10, 11 AND 12. BB, metaphase plates, polar view of spermatogonial divisions, 3, 3 is the third pair of chromosomes.

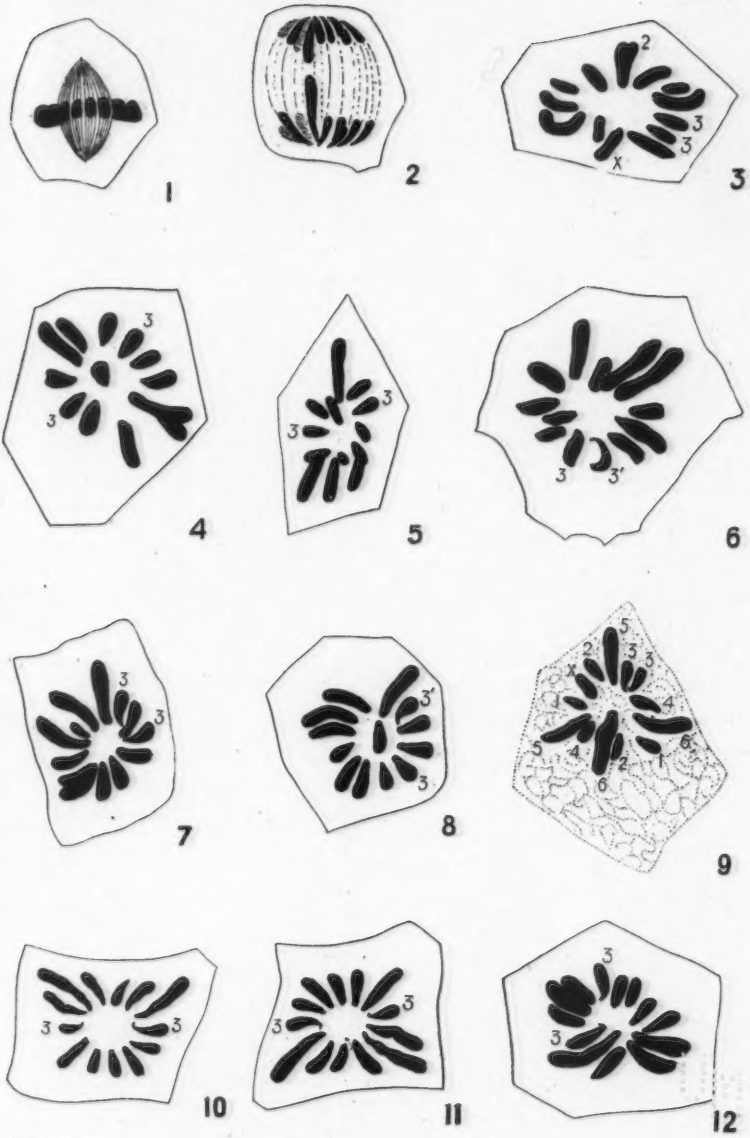

1
2
2
3
3
X
3
3
4
3
3
5
3
3
6
3
3'
7
3
3
8
3'
3
9
5
2
3
3
X
4
5
4
2
1
6
6
10
3
3
11
3
3
12
3
3

PLATE II.

FIG. 13. Early telophase of last spermatogonial division. X, the odd-chromosome.

FIG. 14. Beginning of growth period showing the uniting of the chromosomes to form spireme; X is chromatin of the odd-chromosome in all the figures of this plate.

FIG. 15. Early loose chromatin thread.

FIG. 16. Early contraction stage.

FIG. 17. Later contraction stage and the appearance of the nuclear membrane.

FIG. 18. Large tangled thread.

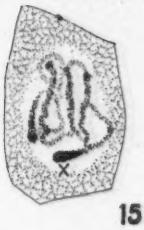
FIG. 19. Beginning of the concentration of the chromatin in various places throughout the thread.

FIG. 20. Breaking up of chromatin thread into the diploid number of chromatin elements.

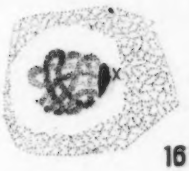
FIG. 21. Further breaking of the chromatin thread and the beginning of the formation of the bivalent chromosomes.

13

14

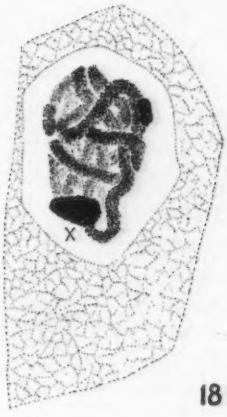


15

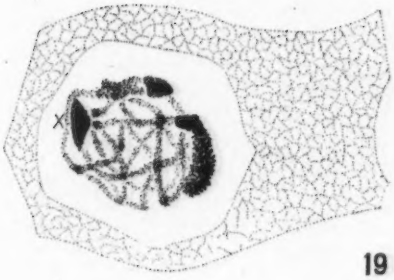


16

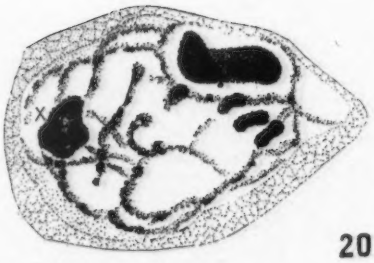
17



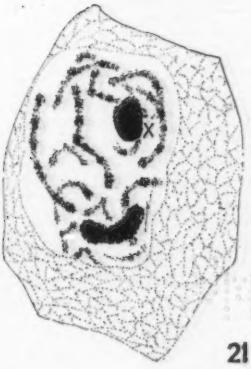
18



19



20



21

M. T. HARMAN.

PLATE III.

FIG. 22. Union of univalent chromatin elements to form bivalent chromosomes. X is the odd-chromosome in all figures of this plate.

FIG. 23. Bivalent chromosomes with the nuclear membrane present.

FIG. 24. Bivalent chromosomes before the formation on the spindle. The two elements of the third chromosome have not yet united.

FIG. 25. First spermatocyte spindle showing the seven chromosomes.

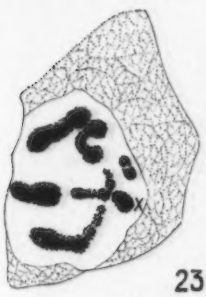
FIG. 26. Metaphase, lateral view of first spermatocyte division.

FIG. 27. Early anaphase of first spermatocyte division. The odd-chromosome approaching one pole in advance of the other chromosomes.

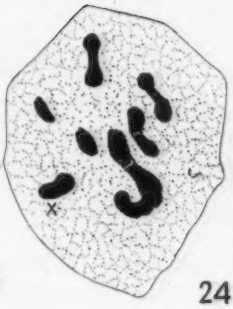
FIG. 28. Metaphase plate, lateral view of second spermatocyte division. Four of the chromosomes show the longitudinal split.

FIG. 29. Metaphase plate, polar view of second spermatocyte division.

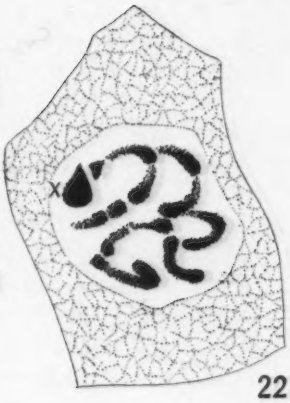
FIG. 30. Metaphase plate, polar view of first spermatocyte division.



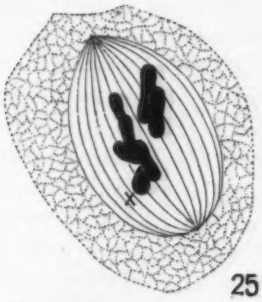
23



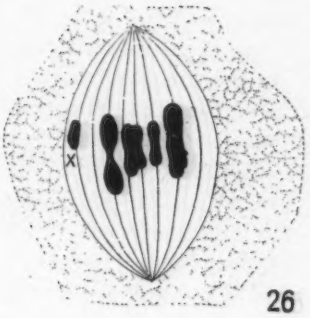
24



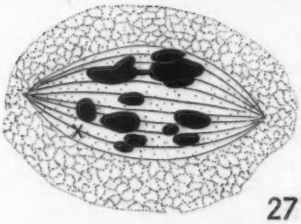
22



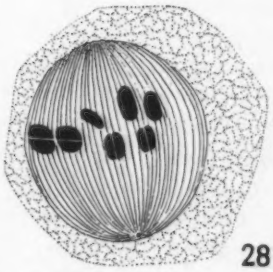
25



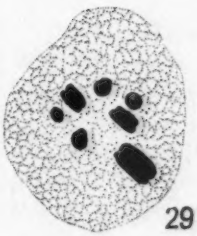
26



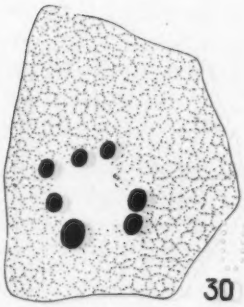
27



28



29



30

WHITE-OCELLI—AN EXAMPLE OF A "SLIGHT" MUTANT CHARACTER WITH NORMAL VIABILITY.

CALVIN B. BRIDGES.

Most of the non-lethal mutant characters of *Drosophila melanogaster* may be described as "slight" in the amount of visible change. This is fortunate, for, in general, the mutant genes that cause great changes in an organ, or that cause several distinct changes in different organs, produce races of poorer viability than the wild type of the species. The amount of disturbance to viability is roughly proportional to the extent of the somatic change. In nature these "extreme" mutant types would not be able to survive in competition with the wild type, and in our experimental cultures their presence leads to aberrant ratios that tend to obscure the simple genetic relations. It is possible to reduce the shortage of these relatively inviable mutant characters only by making the conditions of culture exceptionally favorable. Thus, the number of eggs per culture must be restricted by breeding from a single mother; and the amount of food per culture should be carefully regulated. Great progress has been made in improving the quality and methods of using culture media. While it is possible by such improved methods to make the relatively inviable mutants usable in many kinds of experiments, there remain other experiments that require so high a degree of exactness or the simultaneous use of so many mutants that even one poorly viable mutant is inadmissible. As the number of mutant types increases, we are able to drop the use of more and more of the poorly viable mutants and to replace them by new mutants of normal viability. As already implied, these mutants of normal viability are nearly all characterized by somatic changes of slight degree, or by effects, so far as observable, upon a single organ only. Many of these "slight" characters are perfectly definite in demarkation and offer as great precision of classification as do more extreme mutant characters. Another great advantage of slight mutant characters is that they

do not preclude the simultaneous use of other mutants. On the other hand, the use of such an extreme mutant character as white-eyes prevents the effective use in the same experiment of all other eye colors, and likewise the use of vestigial-wing hinders the use of all other wing and venation characters. The most valuable mutants are, then, those of slight but definite somatic change, free from disturbance to viability or masking effects on other mutant characters.

Origin and Description of the White-ocelli Mutant.

One of the best examples of a slight mutant character that fulfills these conditions is that of "white-ocelli," which was found very early in the breeding work with *Drosophila* (June 21, 1912). The ocelli of wild flies are three small simple eyes in a group on the dorsal posterior part of the head. In color they are of a dilute brownish-red, about that of a coffee infusion. Close examination shows that the color of the ocelli themselves is quite light, about that of weak tea, but that there is a crescent-shaped deposit of dense brownish-red pigment about the median side of the two posterior ocelli and against the posterior side of the anterior ocellus. The apparent color of the ocellus is largely due to this outside pigment seen through the transparent lens-like ocellus, and consequently the color changes in intensity with the angle at which the ocellus is viewed.

It was observed that the ocelli of white and also of vermilion-eyed flies were without color or pigment deposit. These ocellar changes are only other effects of the white and vermilion genes. That the color of the ocelli could vary independently of that of the eyes became apparent when it was found that about half of the flies of the stock of the mutant black-body color had white ocelli, while the remainder had the normal coffee-colored ocelli. Some of the white-ocelli flies were bred together and gave a pure-breeding stock of white-ocelli flies (June, 1912). About a year after this, it was found (July, 1913) that the stock of the third-chromosome recessive spineless was also pure for the white-ocellar color. The gene for white-ocelli was thought to be in the third chromosome, since, in crosses in which the spineless was used, the white-ocellar character generally reappeared in association with the spineless, though sometimes not.

The usefulness of the mutant white-ocelli was not appreciated for some years after its discovery. This neglect was due, in large part, to the fact that the regular examination of flies during this period was carried out by aid of a hand lens only, and the separation of the white from the normal ocelli was difficult because of the small size of the region affected. The later work has been done with a binocular microscope, with special attention to proper illumination and magnification; and under these conditions the separation is complete and entirely accurate, though still somewhat slow.

The Linkage Relations of White-ocelli.

An accurate localization of the gene for white-ocelli was made easy by the use of the two excellent dominant characters dichæte and hairless. The locus of dichæte was near the left end of the map of the third chromosome as then known, being about 13 units to the right of sepia, while the locus of hairless was somewhat to the right of the middle (about 42 units to the right of sepia and 21 to the left of rough). Spineless white-ocelli males were outcrossed to dichæte hairless females; and the F_1 dichæte hairless females were back-crossed by spineless white-ocelli males, with the results shown below:

	0.		1*.		2.		3.		1, 2.		1, 3.		2, 3.		
1918 Sept. 27	D H	ss wo	D ss wo	H	D wo	ss H	D H wo	ss	D ss H	wo	D ss	H wo	D	ss H wo	Total
Total.....	660	528	88	80	116	87	50	53	5	4	2	8	0	1	1,682

* Crossovers in the first region, that between dichæte and spineless, are headed by "1," etc.

The results of this experiment showed that the locus of white-ocelli is to the right of that of hairless by about 6.8 units (a total of 114 crossovers involving region three). The locus of white-ocelli, as thus established, is in what had been the longest unoccupied region of the third chromosome. There had been no workable mutant in the entire distance of about 20 units from ebony (1.5 units to the right of hairless) to rough (21.2 units to

the right of hairless). The ebony rough distance was so great that in constructing a map a correction was required on account of double crossing over. The presence of white-ocelli between ebony and rough gave an opportunity to make a direct test of the amount of double crossing over and consequently of the amount of correction required. The results of the spineless white-ocelli × hairless rough back-cross are given below:

	0.		1.		2.		3.		1, 3.		2, 3.		
1919 Feb. 25	ss wo	H ro	ss H ro	wo	ss ro	H wo	ss wo ro	H	ss H	wo ro	ss	H wo ro	Total
Total.....	1,084	1,126	163	195	118	91	226	255	11	9	0	1	3,179

There was 6.6 per cent. of crossing over between hairless and white-ocelli, which agrees with the value 6.8 found in the previous experiment. Likewise, the white-ocelli rough value of 15.8 is in agreement with the expectation from the usual value of 22 for hairless rough. There was only one double crossover in the hairless white-ocelli rough section—a percentage relatively very low. Comparisons show that in the third chromosome (as in the second) the region near the end of the chromosome has a far lower 'coincidence' than has the mid-region. The amount of correction of the observed crossover value for the hairless ebony interval is thus .063 per cent., or somewhat less than one tenth of one unit. Other back-cross tests involving this region have produced a total of 45,971 flies, of which 19.6 per cent. were crossovers. This value is to be corrected to 19.7, which is the map-distance between ebony and rough.

The Viability of the White-ocelli Mutant.

As just seen, the linkage of white-ocelli was worked out through use of the more convenient spineless white-ocelli stock, while the black white-ocelli stock was discarded. The white-ocelli character persisted in the original black stock. No effort was made to eliminate it, nor, on the other hand, to aid in its survival. In May, 1919, a census of the flies of this black stock showed that approximately half were white-ocellars. That is,

the character had persisted in undiminished frequency from June, 1912, to May, 1919, a period that represented fully 175 generations of flies. During this period the black stock had been carried on in mass-cultures. Every two weeks a new culture was started by transferring, without examination or selection, enough flies to insure breeding. In such mass-cultures overcrowding is extreme, and, in spite of the great numbers of parents, not many more offspring succeed in hatching than hatch from successful pair-cultures. The competition grows keener with the age of the culture, since the number of larvæ is continually increasing from eggs laid each day, while the quantity of available food soon begins to diminish and its quality becomes progressively poorer. The mass-culture method of breeding thus exercises a strong and continuous selection against the perpetuation of the weaker or slower hatching individuals or types. In several instances mixed stocks have been started with equal numbers of different mutations, and this stock transferred without selection through several generations. Watch was kept, and in these cases there has been a progressive change in the composition of the stock, rapid at first, until the numbers of one type were quite small, and thereafter slower but in the same direction. Recessive characters of very low viability may persist for many generations as a small proportion of the population. Their existence is maintained by the inter-crossing of the heterozygotes, whereby the mutant gene escapes the adverse selection that the mutant character suffers. Certain of our mutations are so sensitive to larval overcrowding that the ratios in mass-cultures and in pair-cultures seem to belong to different systems of heredity. Thus, the character strap approaches 1 in 4 in pair-cultures, but may approximate 1 in 16 in sister mass-cultures.

The persistence of the white-ocelli character in undiminished proportion through 175 generations of forced competition means that the mutant is under no disadvantage. Such a mutant might easily survive in nature, and one slightly advantageous might ultimately supplant the original type.

The Modification of Eosin Eye Color by White-ocelli.

An examination of the various stocks of eye color mutations showed that there was a strong correlation between the eye color and the ocellar color. The ocelli of white-eyed flies are entirely colorless. The ocelli of vermilion-eyed flies show a slight trace only of color. Indeed, in the case of vermilion, the vermilion gene has a relatively greater effect upon ocellar color than upon eye color. The ocellar color of pink is so faint that pink can not be used in the same experiment with white-ocelli without some confusion in classification. The ocellar color of the dark eye 'sepia' is itself also darker. In the ten multiple allelomorphs of the white series, the ocellar color is proportional to the eye color. This direct effect of eye color genes on ocellar color suggested that the reverse relation might also hold—namely, that the white-ocelli gene might dilute the eye color. A careful examination of the eye color of white-ocelli flies did not show any certain effect. White-ocelli was crossed to vermilion, and the F_2 vermilion white-ocelli flies were not distinguishable from the simple vermilion flies. In the F_2 of the cross between white-ocelli and eosin, a definite modification of the eosin by the white-ocelli gene was observed. In the case of the males, the eye color of the double form was lighter in intensity and less yellow in tone than that of the eosin brothers. In the females, the change was in the same direction but was less marked in degree. Probably 95 per cent. of the diluted males were separable from the simple eosin, while only about 60 per cent. of the females were thus separable. Eosin is known to be especially subject to specific modification,[1] and the effects of the white-ocellar gene give a color intensity and tone and a sexual difference practically identical with those observed in the case of the modifier 'pinkish.' The gene for pinkish was, however, in the second chromosome, and there are other differences between the two cases.

[1] See *Jour. Exp. Zoöl.*, July, 1919.

OBSERVATIONS ON THE SEXUAL CYCLE OF THE GUINEA PIG.

O. ISHII.

PATHOLOGICAL LABORATORY OF THE WESTERN PENNSYLVANIA HOSPITAL, PITTSBURGH, PA.

In the following work, which was undertaken at the suggestion of Professor Leo Loeb, my aim has been to correlate the microscopical and experimental analysis of the sexual cycle which this author had previously made and further studies which he had planned at that time with a careful study of the cyclic changes as far as they are accessible to the naked eye. These observations were made at the breeding establishment of Miss A. E. C. Lathrop, in Granby, Mass., during the years 1913 and 1914.[1]

THE THREE STAGES OF MENSTRUATION AND HEAT.

1. *Præstrum.*—This period continues for one to one and a half days, during which time the external genital organs become congested and swollen and a slight serous secretion is noticeable. This secretion, while in some instances turbid or viscid, is in the majority of cases more or less transparent; while, as before said, this period averages one to one and a half days, in some instances it may continue for two or three days or more. In the last 4 or 5 hours toward the end of this period a maximum in these changes is reached. This is followed by the period of heat. The duration of the procœstrus period is indicated on the following table. Ninety-four (94) animals served for this observation.

	Length of Procœstrus Period.					
	½ Day.	1 Day.	1½ Day	2 Days.	3 Days.	4 Days.
No. of guinea pigs.....	1	39	34	11	8	1

[1] Accidental conditions greatly delayed the publication of this paper. In the meantime there has appeared a study of Stockard and Papanicolaou on the œstrous cycle of the guinea pig. Notwithstanding this long delay in publication, we believe that our observations present new facts of interest and are worth recording.

On the basis of these observations it may be reasonably said that the average length of this period in an animal of normal physical condition is from one to one and a half day, but after puerperium, or in case of weakness or of pathologic conditions, a shortening or prolongation may be brought about.

2. *Œstrus, or Heat.*—The approach of this period is made apparent by excessive menstrual secretion during the last 4 or 5 hours of procœstrum. This continues for 6 or 7 hours of the œstrus period. This is followed by the secretion of a small quantity of moisture which may be just noticeable, or during the last 3 or 4 hours of the œstrus period menstrual secretion may be lacking altogether; still heat is present in this period.

I give the following data as to the length of period of heat:

	Length of Heat Period.				
	5 to 6 Hrs.	7 to 8 Hrs.	9 to 10 Hrs.	11 to 12 Hrs.	13 to 14 Hrs.
No. of guinea pigs.....	6	51	62	30	9

As can be seen from the above figures, from 8 to 11 hours is the average length of the heat period in the healthy animal. I found those guinea pigs in which the periods were longer (12 to 14 hours) or shorter (5 to 6 hours) to have been lacking in vigor or to have shown abnormal physical conditions. In the normal animal I feel justified in claiming, on the average, 9 to 11 hours as the correct length of this period in the normal guinea pig.

3. *Metœstrum.*—This is the period following the œstrus period and might be considered as the period of recovery from the œstrous changes. The length of this period depends almost wholly on the condition of the animal. In the normal animal 2½ to 3 days are required until the normal condition is re-established. From the figures that follow it will be noticed that the length of this period varies from 1½ to 5 days, but my observations have convinced me that where the recovery is shorter or longer than the average period, the animal is not in a normal healthy condition.

	Length of Metœstrus Period.							
	1½ Days.	2 Days.	2½ Days.	3 Days.	3½ Days.	4 Days.	4½ Days.	5 Days.
No. of guinea pigs....	4	9	30	20	15	10	5	4

I may state that at this period a slightly transparent serous or a turbid or viscid secretion can be noticed; but within 2 or 3 days this will disappear, and the swelling of the external organ will cease and normal conditions will again prevail.

Phenomena of First Menstruation or Heat Period in the Guinea Pig.

The first heat or menstruation of the guinea pig differs in some respects from the later periods of heat. The symptoms of proœstrum are of much longer duration. While as stated above, the normal proœstrum lasts about 1 to 1½ days, during the first menstruation a slight secretion and swelling continues for 3 to 8 days; only then appears the period of heat proper. In some instances the secretion and swelling disappear temporarily. Then a few days later it begins again and now develops into the regular heat. As stated, generally the duration of the proœstrum is variable and usually longer in the first menstrual period.

Diœstrus Period of the Guinea Pig.

The diœstrus period is the length of time between two consecutive menstruations or heat periods. Through observation of the condition of heat we determined the length of the sexual cycle in the guinea pig; we used for this purpose a considerably larger material than Loeb[1] and Stockard and Papanicoloau[2] used in their observations. While our average agrees with that of the aforementioned authors, we found a greater range of variation than Stockard and Papanicolaou. From the observations of Loeb it follows that in some exceptional cases the period may be even shorter than that found by us.

	Length of Time Between Heat.							
	14½ Days.	15 Days.	15½ Days.	16 Days.	16½ Days.	17 Days.	17½ Days.	18 Days.
No. of guinea pigs....	1	40	72	62	36	12	8	1

From these figures we may conclude that the average duration of this period was 15 to 16½ days. I found that where the length of time was decidely shorter (14½ days) or longer (17 to

[1] Leo Loeb, Biological Bulletin, 1914, XXVII., 1.

[2] C. R. Stockard and G. Papanicolaou, *Am. Jour. Anat.*, 1917, XXII., 225.

18 days) than the average, the animals were either in a weak or pathological condition, and that menstrual irregularities subsequently followed. My continuous experiments made on this subject covered both winter and summer of 1913, and the winter of 1914 in Granby, Mass., and during all these seasons, through all temperatures, the same condition prevailed.

General Phenomena of Menstruation and Heat and Pregnancy.

Physical Phenomena of Menstrual Period.

One of the foremost indications of the approach of the period of menstruation or heat is the physical change noticed in the animal. The muscles and joints become tender and relaxed, and by this change it can be determined, whether the animal is normal and healthy. During this period nervousness and a certain mental depression are noticeable; and when the period is over the symptoms most apparent indicate fatigue or exhaustion. However, by the end of the second or third day this fatigue has entirely disappeared and within 1½ to 2 days after cessation of heat a normal animal has regained its full vitality.

Relation between Menstruation and Falling Out of Hair.

I have found, particularly in the guinea pig, rat and rabbit an additional visible sign of heat, viz., the falling out of the hair in unusual quantities during the period of menstruation; as a result of this happening, the external appearance of the animal becomes somewhat glossy. We can determine this by comparing the ease with which the hair can be made to fall off by rubbing the skin in the menstruating animal, on the one hand, and in the infantile, pregnant, amœstrous female or male, on the other hand. In exceptional cases a slight menstrual secretion may develop during pregnancy; in this case the falling out of the hair is slight in accordance with the slight degree of menstrual activity.

Specific Odor of Females during the Period of Heat.

Many (but not all) males can evidently clearly distinguish between females which are in the period of heat on the one hand and those in which there is merely menstrual secretion and in

which both are lacking on the other hand. Therefore the male attempts copulation only in the former case. When a number of animals of both sexes are together and among them is a female in the heat period, the males run around and search for that particular female, ultimately finding it.

I am convinced from this searching of the male for the female in heat that he is led to do so by a special odor characteristic of the female, when it reaches the heat period. This peculiarity is common to the mouse, the rat and the rabbit.

Influence of Food upon the Character of Heat and on the Time when Sexual Maturity is Reached.

The vigor of the heat in guinea pigs depends upon the nourishment which the animals have received. Guinea pigs which had been poorly fed develop weak heat. This applies to the duration of the heat as well as to the vigor of the symptoms. The length of the diœstral period does on the other hand not seem to be affected by the state of nourishment.

I made observations on the influence of food on the time when sexual maturity is reached.

I. On especially prepared rich food the first heat was observed 45 to 60 days after birth.

II. On usual food 55 to 70 days after birth.

III. On food of poor quality 75 to 100 days after birth.

The usual food consisted of hay (or grass), carrots (or beets) and oats. In the rich diet corn cake was added, while the poor diet consisted merely of hay (or green grass) and oats. When the quality of the food was above the ordinary, its effect on the size of the guinea pig as well as their physical condition in other respects could be seen. They were of the average condition, when ordinary food was given at regular intervals, and the poorly fed animals were poorly developed physically. The food is therefore of the greatest importance as far as the time of sexual maturity and the character of the heat are concerned.

Influence of Climate on the Character of the Heat and the Length of Sexual Cycle in the Guinea Pig.

The extreme temperature of the summer months (a temperature of over 90° F. in Granby, Mass.) has a weakening effect on

the guinea pigs. And the heat is therefore not so vigorous as it would be under other climatic conditions. The length of the diœstrous period on the other hand seemed to be the same during the warm and cold season of the year.

Does Contact with the Male Influence the Time of Appearance of the First Heat?

If young female guinea pigs are kept in a cage with males and other guinea pigs of the same descent are kept separated from males, the first heat appears in both lots at the same age. This shows that psychical factors such as the stimulation of the male does not influence those conditions which lead to the first heat.

The Copulation-reflex of the Heat Period and the Diagnosis of the Heat Energy.

This reflex can be elicited by the male guinea pig seeking copulation, when it touches or gently massages with his forefeet the lumbar portion of the spinal column of the female which is in heat. The female will then lift up the tail and the vagina will be opened in preparation of the copulation. The same reflex can be called forth if an observer touches with his finger the same part of the body of the female guinea pig. The readiness and vigor with which the reflex occurs can serve as a measure of the energy or vigor of the heat.

Menstruation during Pregnancy.

I very carefully watched twenty guinea pigs during their state of pregnancy for a sign of menstruation and found in ten no evidence of menstrual secretion, but in the remaining ten there was some secretion present; not all of those however showed signs of heat. The menstrual period is not definite during pregnancy. Statistically my results were as follows:

Menstruation was noticed in 3 guinea pigs 27 days after copulation
" " " " 3 " " 30 " " "
" " " " 2 " " 40 " " "
2 " " had menstrual secretion twice, the first time 15 days and the second time 30 days after copulation.

While in these cases the swelling of the external genitalia and the secretions are manifest, they are slight. The secreted material

is mostly transparent, but sometimes turbid. It continued for 3 to 8 days in my observation; then the external genitalia returned to a normal condition.

Some authors suppose that the diagnosis of the menstrual secretion is very difficult in the guinea pig; however careful observation has shown me that the diagnosis can be readily made and hardly misinterpreted.

Diagnosis of Pregnancy about 14 to 15 Days after Copulation.

About 14 or 15 days after copulation we can correctly determine the success or failure of the impregnation. If at that time the external genitalia are in a normal condition we may almost always assume a successful pregnancy. If, on the other hand, there should be within that time a noticeable swelling of the external genitalia, accompanied by secretion, this may in all cases be taken as an indication of failure. As we have stated above, the same phenomenon may be present, perhaps in a milder form, at this period of pregnancy, but this is a very rare occurrence; and as a general rule in the case of failure (early abortion) the secretion and swelling is much more marked.

The Sexual Cycle in Cases of Early Abortion.

In cases of early abortion I found the period of heat in some cases somewhat accelerated and more irregular than in normal animals.

DIOESTRUM.

	Length of Heat Following Copulation.				
	12½ Days.	13½ Days.	14½ Days.	15½ Days.	16 Days.
No. of guinea pigs examined	1	2	2	3	5

As shown in the table, the heat period may appear as early as 12½ or 13½ days after copulation. In control cases the shortest period observed was 14½ days and this occurred only once; and, as I stated above, I believe that in normal cases 15 days represents the minimum and that the shorter periods are usually found in cases of abortion.

The Length of the Period of Gestation in the Guinea Pig.

On the basis of our observations we can give the following statistical data concerning the length of the period of pregnancy in the guinea pig.

	Length of Gestation Period.							
	61 Days.	62 Days.	64 Days.	66 Days.	67½ Days.	68 Days.	69 Days.	71 Days.
No. of pregnant guinea pigs	2	2	4	4	8	15	5	1

The shortest period was 61 days; but those young animals which are born 61, 62 and 64 days following copulation show a certain lack of development; they are really born prematurely and in the majority of cases they soon die, although they may be able to survive. Those born 66 days after copulation usually lived and continued to grow, but those born from 67 to 69 days following copulation show the maximal development after birth; these have the best chance to develop into absolutely healthy, perfect animals. We may therefore conclude that 68 days is the regular period of gestation in the guinea pig.

Separation of the Symphysis Pubis in the Guinea Pig at the End of Pregnancy, at the Time of Birth and During the Period Directly Following Birth.

Through examination with the finger we discern in the guinea pig a gradual separation of the symphysis pubis which begins about 61 to 63 days after copulation and increases as the time of labor approaches to a width of 7 to 8 millimeters or more; but within one or one and a half days after birth the symphysis pubis assumes again its normal condition. The following table shows the time when the separation becomes apparent:

	Beginning Separation of Symphysis Pubis.		
	61 Days, Following Copulation.	62 Days, Following Copulation.	63 Days, Following Copulation.
No. of pregnant guinea pigs.	5	5	4

The length of time from the beginning of the separation of the symphysis to the onset of labor is shown on the following table:

	Duration of Period of Separation of Symphysis Pubis Prior to Birth.		
	4½ Days.	5 Days.	7 Days.
No. of pregnant guinea pigs.....	1	6	7

The following table shows the length of time necessary for the symphysis pubis to return to its normal condition following labor:

	Time when the Symphysis Pubis has again Become Normal Following Labor.			
	½ Day.	1 Day.	1½ Days.	2½ Days.
No. of guinea pigs.....	4	8	5	1

From these data we may conclude that the separation of the symphysis pubis begins from 5 to 7 days previous to birth, or from the 61st to the 63d day after copulation. Under ordinary conditions birth should not take place before the 5th day following this separation. The length of the period of gestation should therefore usually be 68 days.

Preparation for Labor.

In the guinea pig the separation of the symphysis pubis serves as a preparation for parturition, inasmuch as the orifice of the normal pelvis is too narrow to permit birth to take place. Moreover we find at the same time the same remarkable softness and elasticity of the muscles and joints as during the ordinary menstrual period. This same effect (emanating from the ovary or pituitary gland) I have also noticed in the rat and in the rabbit and I presume it is the same in all mammals.

The Time of the First Heat in the Puerperium.

The time of the beginning of heat in the period directly following labor is somewhat variable. In 20 guinea pigs in which parturition set in at the normal time (67 to 69 days after copulation) I found the following figures:

	Beginning Puerperal Heat After Parturition.				
	Soon After	3 Hours After.	5 Hours After.	7 Hours After.	No Heat.
No. of examined guinea pigs	7	7	3	1	2

As our table shows, in 2 out of 20 guinea pigs no heat at all was observed at this period. There was evidence pointing to the conclusion that the heat is less vigorous at this stage than in the normal menstrual period. On the other hand the heat seems perhaps to be of a somewhat longer duration at this period as shown in the following table:

	Duration of Puerperal Heat.		
	7 to 9 Hrs.	10 to 11 Hrs.	12 to 13 Hrs.
No. of guinea pigs.....	8	7	7

We find therefore that in about one third of the guinea pigs at this period the average duration of heat was 12 to 13 hours, while in normal animals the heat continues for that length of time only in a small minority of cases. Whether or not heat occurs in the period directly following parturition depends upon the duration of the period of gestation as shown in the following table:

	Duration of Pregnancy.							
	61 Days.	62 Days.	64 Days	66 Days.	67½ Days.	68 Days.	69 Days.	71 Days.
No. of observed guinea pigs.	2	2	4	4	8	15	5	1
Puerperal heat.....	−2	−2	−4	−1/+3	−1/+7	−2/+13	+5	+1

+ coming in heat; − not in heat.

This table shows that heat does not occur in the period directly following parturition in cases in which the duration of pregnancy was less than 66 or 67 days. In cases in which the duration of pregnancy was less, birth occurred prematurely and the phenomena of the puerperium had not yet fully developed at the time of the completion of labor. If in cases of parturition occurring 61 or 62 days after copulation the first heat following parturition would take place 6 or 8 days later, the first heat would correspond to a typical puerperal heat, provided the duration of pregnancy would have been 68 or 69 instead of 61 days.

On the Duration of the Sexual Cycle following Parturition.

The time of the second menstruation in the period following parturition is rather variable as shown in the following table:

	Development of Second Menstruation Following Parturition.									
	15½ Days.	16 Days.	16½ Days.	17 Days.	17½ Days.	18 Days.	21 Days.	22 Days.	23 Days.	Total.
No. of guinea pigs.....	2	1	4	5	2	3	1	1	1	20

Among 20 guinea pigs in the period following the puerperium I found complete menstruation and heat in 15 cases, while in 5 animals I found only menstrual secretion without heat. The majority of these animals suckled their young and among those the deviation from normal was somewhat less; in these cases the duration was mostly 15½ to 18 days.

Effect of Lactation and Non-Lactation on the Periodicity of the Sexual Cycle after Parturition.

In cases in which the young were suckled the next period of heat took place approximately at the normal time and the conditions continued from then on in a normal manner. It was different in cases in which bodily weakness of the mother prevented the suckling of the young. In the latter cases the return of menstruation was delayed so that it occurred as late as 21 to 23 days after parturition. In the normal suckling animal the sexual periodicity following labor is therefore similar to that of the normal animal, although certain minor variations depending on the vitality of the animal occur even in such cases. The second or third menstruations occur usually after the regular interval of 15 to 16 days even in the weak animal. In the latter, however, we may at these times instead of heat merely find menstrual secretion.

The Effect of Suckling on the Mother and the Young.

Marshall[1] expresses the opinion that in the young separated prematurely from the mother growth will continue. My own observations lead me to a different conclusion. I have found

[1] F. H. A. Marshall, "The Physiology of Reproduction." London, 1910.

that if a mother suckled three young, the growth of the latter was very slow, or death resulted; in addition the weakening effect on the mother was quite noticeable; it sometimes caused her death, and in most cases the death of the young occurred. If the young died, the mother soon regained her health and strength. The result will therefore be disappointing either as far as the condition of the mother or of the young is concerned. It has been my experience that when the young were taken away from the mother within 15 or 20 days after birth and the ordinary vegetable and oat diet was given them, development was very slow and in most cases death occurred. In any case the young to attain healthy growth should not be taken from the mother until more than 25 to 30 days of age. Under natural conditions they cease suckling after 35 to 40 days, when they seek their own food. I am therefore of the opinion that a litter of three very seldom shows normal development when suckled by one mother.

When I combined one mother with two and another with three young, each mother suckled on the average 2½ young. In such cases I obtained good results. While two or three young is the average size of a litter, I have known the number to vary from one to six; the latter number, however, only occurred once in my observation. When but one young is born, it is usually well developed and vigorous; but to be successfully raised it must be suckled for the usual period or at least for a time approaching it. There is another symptom in addition to the bodily weakness, which the mother shows as the result of too intensive suckling: it consists in the changing of the color of the pupil of the eye which becomes pale or white. It may be associated with general exhaustion.

Under what Condition is Copulation followed by Pregnancy?

As we stated above the normal duration of heat is about 10 hours. If copulation took place within 3 or 4 hours after the beginning of heat, pregnancy followed only rarely. If the copulation took place at a later period of the heat, better results were obtained. According to my observations pregnancy occurred in about 73 per cent. to 80 per cent. of the cases. In such cases in which copulation was not followed by pregnancy a second or

third attempt during the next two periods of heat were mostly successful.

Sobotta[1] states that the guinea pig in captivity can become pregnant more frequently in summer than in winter. My observations do not bear out this statement. In my experience the pregnancies resulted almost as often in the cold as in the hot season. The winter climate is better adapted for the guinea pig in captivity than the hot summer weather when the temperature is liable to exceed 90° F. Under those conditions the animal suffers and becomes poorly developed as the result of undernourishment. On the other hand, it is almost always in good condition in winter. Therefore the cold season agrees better with guinea pigs than the hot season under conditions of captivity. In exceptional cases the guinea pig may become in winter time too fat to be favorable for impregnation; and this may even happen in summer time.

Relation between Growth, the First Period of Heat and the Period of Sexual Maturity in the Guinea Pig.

In my observations the weight of the guinea pig is ordinarily three ounces soon after birth. However, the weight of the young is in accordance with the age of the mother and also the number of the litter. As a rule the young born from a young mother are small in size and in litter. On the other hand, when the mother is older than 5 months, the weight of the young is mostly larger. For instance, in one litter the young weighed 6 or 7 ounces soon after birth.

Minot[2] has concluded that the guinea pig is slow in growing and that it attains its full size one year after birth. My observations are as follows: The young guinea pig at the time of the first heat was about two months old; about 7 to 10 days before the beginning of heat, the growth almost stopped or at least progressed very slowly. At the time of the first heat period the average weight was 11 to 13 ounces. After the first heat period had passed it began to grow very rapidly. About two or three

[1] Sobotta, *Anat. Hefte*, 1906, XXXII.

[2] Minot, C. S., "Growth and Senescence," *Journ. Physiol.*, 1891, XII., 97. "Problems of Age, Growth and Death," *Popular Science Monthly*, 1907, LXX., 481, and LXXI., 97 and succeeding numbers.

months later, at the age of 4 to 5 months, it attained its full weight, about 20 to 28 ounces, but one exceptional animal weighed 34 ounces in a non-pregnant condition. However, if we feed a special diet of nutrient material and the animal is otherwise well taken care of, then the growth progresses rapidly and the heat begins earlier than usual. The guinea pig which weighed 34 ounces was fed on the usual farm or laboratory food. Therefore we must remember that the growth of the young depends considerably upon the character of the food.